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Antimicrobial finish on cotton fabric using *Harsingar*

Sandeep Bains, Rajdeep Kaur and Manisha Sethi

Abstract
Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable health-care regime and are an important source of livelihood for indigenous and rural populations. *Nyctanthes arbor-tristis* commonly known as Night Jasmine and *Harsingar* is an important traditional medicinal plant. The present study deals with preliminary phytochemical screening and quantification estimation of bioactive constituents like phenolics, flavonoids, alkaloids, tannins, terpenoids and proteins using standard methods. The extraction of the plant source was done using ethyl alcohol, methyl alcohol and distilled water. Presence of major phytochemicals like Phenolics, Flavonoids and Alkaloids had been detected in the extract whereas quinones, anthraquinones and carboxylic acids were found to be absent in the plant extract. It was observed that yield of extract was more (46ml/50 ml) when the leaves of the plant was extracted with distilled water while it was 44ml/50 ml when extracted with 70% ethanol. The total phenolic content of *Harsingar* was 208.25 mg when extracted with 70% ethanol and 107.33 mg with distilled water. The antibacterial activity of the plant was recorded. When the plant source was treated against both *S. Aureus* bacteria and *E. Coli* bacteria, with extraction using 70% ethanol, maximum zone of inhibition (16 mm and 7 mm respectively) was noticed. The findings of the study also revealed that ethyl alcoholic extract was more effective than aqueous extract for anti bacterial finish when applied on the cotton fabric.

Keywords: Alkaloids, antibacterial, antifungal, medicinal, phytochemical screening

Introduction
Medicinal plants contain some organic compounds which provide definite physiological action on the human body. This is due to the presence of bioactive substances which include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These phytochemical compounds are synthesized by secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes has been shown to have inhibitory effects on all types of microorganisms. Plants and their products have been part of phyto-medicines since time immemorial. These can be derived from barks, leaves, flowers, roots, fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances used as antibacterial agents.

The use of natural products for antimicrobial finishing of textiles has opened up new avenues in the field of technical textiles. Although there are many natural sources rich in antimicrobial agents, the study on their use in textiles is very limited and not documented. The major challenges in application of natural sources for textile application are that majority of the sources are complex mixtures of several compounds and also the composition varies in different species of the same plant [1]. However, because of their eco-friendly nature and non-toxic properties, they are still considered as novel means for niche applications such as medical and health care textiles. Thus, Nyctanthes arbor-tristis an ornamental/ flower species was selected for the study.

Nyctanthes arbor-tristis belonging to family Oleaceae is a small sacred ornamental tree

commonly known as Parijata (Sanskrit) and Harsingar (Hindi), Night Jasmine (English). The generic name 'Nyctanthes' has been coined from two Greek words 'Nykhta' (Night) and 'anthos' (flower) [2, 4]. It is a small tree with its fragrant flowers found wild in the forests of Central India and Sub-Himalayan regions, cultivated in gardens in many parts of India. This plant has several medicinal properties. Different parts are used traditionally for treatment of various diseases like sciatica, chronic fever, skin disease and possess properties like anti-inflammatory, hepatoprotective, antioxidant etc. The plant based traditional knowledge has become a recognized tool in search for new sources of drugs.
Environment friendly natural products for textile application are gaining interest, worldwide. The relatively lower incidence of adverse reactions occurring from natural sources coupled with their reduced cost can be exploited as an attractive eco-friendly alternative to synthetic agents for textile wet processing. Hence the present study was designed with the objectives to characterize the plant extract for antimicrobial activity, standardize a protocol for application of antimicrobial finish and to assess the performance of treated fabrics.

Material and Methods

Processing the plant source: The plant leaves were collected and washed in RO water. These leaves were dried in hot air oven at 40±1°C. The dried plant source was powdered in a blender and was refrigerated.

Extract preparation: The plant source powder was soaked in distilled water, ethyl alcohol and methyl alcohol separately for 24 hours in three beakers. The supernatants were centrifuged and filtered. The Total Phenolic content (TPC) was tested using a UV Vis Spectrophotometer. Agar well diffusion method was used for screening the extracts for gram positive and negative bacteria. Further, the antifungal activity of the herbal extract was also studied.

Phytochemical analysis: Chemical tests for the screening and identification of bioactive chemical constituents of the medicinal plants under the study were carried out for extracts using the standard procedures. For each procedure, details of these have been furnished below:

Test for alkaloids: The presence of alkaloids in the plant extract was tested using Mayer's reagent and Dragendroff's reagent. For this, two milliliter of the plant extract was treated with lml of Mayer's reagent. Dull white precipitates indicated the presence of alkaloids. Similarly, two milliliter of the extract was treated with 1ml of dragendroff's reagent. Formation of orange red precipitates indicated the presence of alkaloids.

Total flavonoid content (TFC): TFC is determined by aluminium chloride colorimetric method using rutin as reference standard. Aliquots (1ml) of appropriately diluted extracts were pipetted into test tubes and mixed with 0.15ml of 5% $NaNO_2$. After 5 minutes, 0.15ml of 10% $AlCl_3$ solution was added and the mixture was allowed to stand for 5 minutes, and then 1ml of 1M NaOH was added. The reaction solution was well mixed, kept for 15min and the absorbance was determined at λ415nm using the UV-Visible Spectrophotometer. TFC was expressed as rutin equivalent (RE) in milligrams or micrograms per gm sample.

Total Tannin Content: Total tannin content was estimated by Folin-Denis method. Solution of the extract was made with distilled water and 3 drops of 5% ferric chloride were added in solution. A green-black colour indicated the presence of tannins. Tannic acid was used as the reference standard. The results are expressed as mg/g of Tannic acid equivalent (TAE).

Test for saponins: Half a gram (0.5 g) of each extract was placed in a test tube and then 0.5 ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15 mins indicated the presence of saponins.

Test for Terpenoids: Salkowski's tests were used to see the presence of terpenoids in plant extracts. The extract was dissolved in l ml of chloroform and equal volume of concentrated sulphuric acid poured by sides of the test tube. The upper layer turning red, the sulphuric acid layer becoming yellow with green fluorescence represented the terpenoids in the extract.

Total phenolic content (TPC): TPC was determined by the Folin-Ciocalteu assay method, using gallic acid as the reference standard. To 1ml of solvent extract 100µL of Folin-Ciocalteu reagent was added and incubated at room temperature for 3 minutes. Then 2ml of 10% Na_2CO_3 solution was added to the mixture. The resulting solution was incubated for 60 minutes at room temperature under dark conditions, the absorbance was measured at λ765nm using the UV-Visible Spectrophotometer. TPC was expressed as gallic acid equivalent (GAE) in milligrams or micrograms per gm of sample.

Antibacterial activity assay: The antibacterial activity of different plant extracts was determined by the Disc Diffusion Method.

Preparation of disc: Sterilized discs (6mm in diameter) 20—25 were soaked in different concentrations 3, 5, 7, 9 gpl) of each plant extract for 24 hours at the room temperature. Next day discs were dried at room temperature under sterile conditions.

Preparation of culture broth: The cultures used for the broth were - *Escherichia Coli* negative MTCC 443 and *Staphylococcus aureus* positive MTCC 737. Pure culture of *E. coli* and *S. aureus* were inoculated in the nutrient broth and incubated at 37°C overnight. The turbidity of the above culture was adjusted equivalent to 0.5 Mac Farland standard by taking absorbance at 430nm.

Antibacterial Activity: The antibacterial activity of the sample was evaluated using agar disc diffusion assay. Briefly, a 24 and 48 hours old culture of selected bacteria was mixed with sterile physiological saline (0.9%) and the turbidity was adjusted to the standard inoculums of Mac Farland scale 0.5 (106 colony forming units (CFU) per ml). Petri plates containing 20 ml of Nutrient Agar was used for antibacterial activity. The inoculum was spread on the surface of the solidified media and What man No. 1 filter paper discs (5 mm in diameter) impregnated with the sample (20µl/disc) were placed on the plates. Streptomycin was used as positive control for bacteria. Plates inoculated with the bacteria were incubated for 24 hour at 37° C. The diameter of zone of inhibition was measured in millimeters (zoi-mm).

Antifungal Activity: Poisoned technique was performed to investigate antifungal effect of sample extracts against *A. niger*. The extract was tested for the antifungal activity, the fungi employed for screening was sub-cultured using potato dextrose agar medium. The potato-dextrose-agar medium was sterilized by autoclave at 121°C (15 lb/sq. inch), for 15 minutes. The petri-plates, tubes and flasks plugged with cotton were sterilized in autoclave at 121°C, for an hour. In each sterilized petri-plate (10 cm diameter), about 30 ml each of molten potato dextrose-agar medium was inoculated with fungus (5 mm disc of the fungus grown) and transferred, aseptically. The petridishes were incubated at 28± 1˚C temperature and the diameter of the zone of inhibition was read using an 'antibiotic zone reader'. Metalaxyl was used as the positive control. All the results obtained were expressed as % inhibition (%I).

Antioxidant Activity (DPPH Radical Scavenging - IC50): Radical-scavenging capacity of sample extracts were estimated according to the procedure reported procedure using the DPPH radical [3]. In brief, an aliquot (1 ml) of sample extract was mixed with the freshly prepared 1mL of (200 μM) DPPH in ethanol. The control contained all the reagents except phenolic extracts. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured using UV-Vis Spectrophotometer. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{Scavenging \%} = \frac{\text{(Absorbance of standard - Absorbance of sample)}}{\text{Absorbance of sample}} \times 100$$

The results obtained were represented as IC50* value.
*****IC50-the concentration required to scavenge 50% of free radicals under experimental conditions

Results and Discussion

The extract was processed (dried and powdered) and tested for various phytochemical analysis. It was observed from Table 1, that the yield of extract of *Harsingar* leaves was more (46ml/50 ml), when extracted using distilled water while it was 44.1ml/50 ml when extracted using methanol and 44ml/50 ml when extracted using 70% ethanol. The total phenolic content of *Harsingar* was 208.25 mg when extracted with 70% ethanol, 196.03 mg with methyl alcohol and 107.33 mg with distilled water. Amongst the tested solvents, the highest values in total phenolic content, total flavonoid content, total carotenoid content, total tannin content, total anthocyanin content and antioxidant activity were detected in the following order: Ethyl alcohol >Methyl alcohol > Distilled water in the plant source.

Table 1: Quantitative Analysis of Phytochemicals present in medicinal plants

Constituents	Units	Ethyl alcohol	Methyl alcohol	Distilled water
Yield	ml/50 ml	44	44.1	46
Total Phenolic Content	mg/g GAE	208.25	196.03	107.33
Total Flavonoid content	mg/g RE	123.13	112.28	34.58
Total Carotenoid Content	mg/g βcarE	453.19	428.72	98.11
Total Tannin Content	mg/g TAE	3.44	1.40	NI
Total Anthocyanin Content	mgCy3Glu/ml	68.32	62.21	8.99
Antioxidant Activity	DPPH scavenging activity (IC50)	49.23	48.55	11.45

1mg/g GAE – milligram per gram Gallic acid equivalent
2mg/g RE - milligram per gram Rutin equivalent
3mg/g βcarE - milligram per gram β-carotene equivalent
4mg/g TAE - milligram per gram Tannic acid equivalent
5mgCy3Glu/ml - milligram cyanidin-3-glucoside per ml
5DPPH scavenging activity (IC50) - $\frac{\text{Absorbance of Control - Absorbance of Sample x 100}}{\text{Absorbance of Control}}$

Lack of zone of inhibition does not necessarily mean an absence of activity. A zone is generally shown by antimicrobial agents that are 'leaching type', *i.e.*, they leach out of the fabric and kill the microbes present on as well as around the treated fabric. Generally, the bigger the zone, the higher is the antibacterial activity[1]. The antibacterial activity of the plant was recorded. The results of antimicrobial activity of the herbal plant extract are presented as zone of inhibition in Table 2. The gram positive bacteria chosen for the study was *Staphylococcus aureus* and gram negative bacteria was *Escherichia Coli*.

Table 2: Antimicrobial activity (Zone of inhibition) of the plant extract

Attributes		Ethyl alcohol	Methyl alcohol	Distilled water
Antibacterial (mm)	S. Aureus	16	16	10
	E. Coli	7	5	NI
Antifungal (mm)		9	9	2

NI – No zone of inhibition

When the plant source was treated against both *S. Aureus* bacteria and *E. Coli* bacteria, with extraction using 70% ethyl alcohol, maximum zone of inhibition (16 mm and 7 mm respectively) was noticed. The zone of inhibition was 10 mm, when extracted in distilled water with *S. aureus* bacteria, while there was no zone of inhibition when the sample was treated using distilled water with *E. coli* bacteria. While recording the Antifungal activity of the plant it was found that when treated against *A. Niger*, extracted using both 70% ethyl alcohol and methyl alcohol gave zone of inhibition of 9 mm, while it was 2mm when extracted using distilled water.

Presence of major phytochemicals like alkaloids and flavonoids have been detected in the extract using ethyl alcohol and methyl alcohol. Whereas, quinones, anthraquinones and carboxylic acids were found to be absent in the plant extract.

Table 3: Qualitative Analysis of Phytochemicals present in medicinal plants

S. No	Solvents	Name of test	Harsingar leaves		
			Ethyl alcohol	Methyl alcohol	Distilled water
1.	Alkaloids	Dragendorff Test	++	++	+
		Wagner test	++	++	+
2.	Flavonoids	Ammonia Test	++	++	+
		Sodium hydroxide test	+	+	_
3.	Tannins	Ferric chloride test	++	+	+
		Gelatin test	+	+	_
		Lead acetate test	+	+	_
4.	Saponins	Foam Test	_	_	_
5.	Terpenoids	Salkowski Test	+	+	_

(*+ Present, - Absent of the particular compound*)

The notations, +++, ++, + and – refer to appreciable amounts (positive within 5 min); moderate amounts (positive after 5 min but within 10 min); trace amounts (positive after 10 min but within 15 min) and completely absent, respectively.

As evident from table 3, it can be observed that using distilled water, flavonoids were absent with sodium hydroxide test, tannins were absent with the gelatin and lead acetate test, terpenoids were absent using salkowski test. Further, saponins were absent in all the three mediums using foam test.

The DPPH scavenging activity has been used to detect antiradical activity of samples. The stable radical, DPPH, has a λ max at 517 nm and could readily undergo scavenging by antioxidants. Higher free radical scavenging activities of samples was indicated by lower absorbance at 517 nm. Amongst the tested solvents, the highest radical scavenging activity was detected in ethyl alcohol. It was observed that that ethyl alcoholic extract was more effective than aqueous extract for anti microbial finish when applied on the cotton fabric.

After studying these properties, the extract was applied to the fabric. Extracts of the plant source in different concentrations (5%, 10% and 15%) were applied on 100% cotton fabric with GSM 126, the count of the fabric being Warp 51, Weft 38 by direct and pad dry cure method. Citric acid was used as a cross linking agent. The fabric was dipped in the solution of 4% citric acid for 30 minutes. The fabric treated with citric acid was dipped in different concentrations for half an hour. The impregnation of extract into fibre was carried out using padding mangle. The procedure was repeated twice to ensure proper impregnation of the extract.

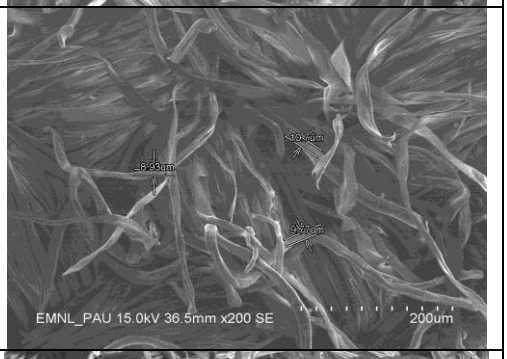
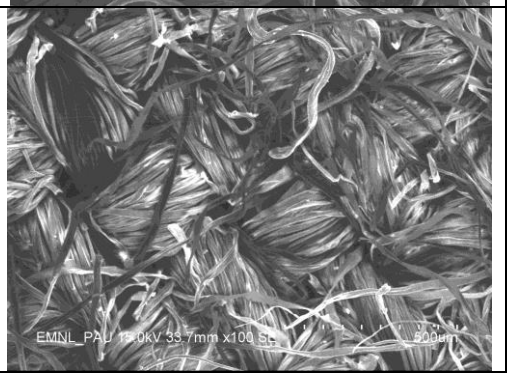
Extract Percentage	SEM images
5%	
10%	
15%	

Plate 1: SEM images of fabric finished with *Harsingar* leaves extract

The SEM was carried out to know about the properties of the finished fabric. The surface morphological studies using SEM show the surface coating and some fibrillation (Plate 1). Using 4% citric acid as cross linking agent, the fibre thickness ranged between 8.93 µm to 10.7 µm.

Table 4: L*a*b* values of finished fabric

Extract Percentage	L*	a*	b*	K/S value
5%	91.01	-1.33	-4.49	36.99
10%	90.32	-1.79	-1.74	45.74
15%	89.34	-2.44	0.27	46.49

The L* value indicates the darkness and lightness of the fibre/ fabric thus the results from the Table 4 reveal that with the increase in extract percentage, the L value decreased which indicates darkness on the surface of the fabric. The negative a* value indicates the green colour of the fabric. Thus the values in the table indicate that the fibre was not much red in hue. The positive b* value indicates the yellowness of the fibre when fabric was treated with 15% plant extract. The K/S value was maximum (46.49) with 15% extract showing maximum colour strength.

Conclusion

The use of natural products for antimicrobial finishing of textiles has opened up new avenues in the field of technical textiles. Although there are many natural sources rich in antimicrobial agents, the study on their use in textiles is very limited and not documented. However, because of their eco-friendly nature and non-toxic properties, they are still considered as novel means for niche applications such as medical and health care textiles. Hence the present study was designed with the objectives to characterize the extract from *Harsingar* leaves for antimicrobial activity, standardize a protocol for application of antimicrobial finish and to assess the performance of treated fabrics.

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