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Assessment of total phenolic content and antimicrobial activity of plants leaves extract

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Abstract
Novel finishes of high added value for apparel fabrics are also greatly appreciated by a more discerning and demanding consumer market. Antimicrobial textile with improved functionality finds a use in variety of applications such as health and hygiene products. Now plant resources are getting attention of manufacturer, academician and researchers due to its ecofriendly nature and versatile properties such as antibacterial and ultra-violet protection which can be used for imparting finishing to textiles. In the present study the efforts were made to impart antibacterial finish to the cotton textile using natural resources. Here the leaves of ten plant sources *viz. neem, safeda, aonla, sagun, nimboo, jamun, curry patta, peepal, mehandi* and *bael* were taken on the review basis and their yield count in ethanol and methanol extract, Total Phenolic Content (TPC) and antibacterial properties against *S. aureus (gram +)* and *E. coli* (gram -) bacteria and antifungal properties against *aspergillus niger* fungus were studied using qualitative test method. It was found that jamun leaves showed the highest yield in aqueous extract whereas as aonla showed the highest yield in ethanolic extraction medium. The Total Phenolic Content (TPC) was found highest in neem leaves in both medium of extraction i.e. aqueous and ethanolic. It was also noticed that ethanolic medium of extract showed maximum resistance against bacterial as well as fungal growth as compared to aqueous extraction medium. In etanolic medium of extraction, the *safeda* leaves showed the highest zone of inhibition i.e. 23 and 20 mm against *S. aureus (gram +)* and *E. coli* (gram -) bacteria, respectively followed by *bael* leaves (20 mm each) where as neem leaves showed the highest zone of inhibition i.e.11 mm against *Aspergillus niger fungus* in ethanolic medium of extraction.

Keywords: Antibacterial properties, total phenolic content, leaves, extract

Introduction
For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of plant compounds for pharmaceutical purposes has gradually increased [1]. Medical textiles are a rewarding and an exciting area having enormous possibility to re-model people's daily lives. Use of textiles in healthcare industry goes back to centuries before Christ. Use of cotton, silk and flax etc., for a variety of purposes like wound dressings, sutures, dates back to 5000 BC [2]. The growth of microorganisms on textiles inflicts a range of unwanted effects not only on the textile itself but also on the wearer. These effects include the generation of unpleasant odor, stains and discoloration in the fabric, a reduction in fabric mechanical strength and an increased likelihood of contamination [3]. For these reasons, it is highly desirable that the growth of microbes on textiles be minimized during their use and storage [4].

The antibacterial finish treatment has become vital area of medical textiles due to its use in surgical and healthcare activities performed against potential pathogenic microorganisms present in hospital environment and cause cross-infection diseases.[4] Hence to protect the wearer and also the fabrics from infection, textile fabrics can be finished with different antimicrobial agents. Antimicrobial textiles can improve functional properties like infection control, barrier material and can also be used in a variety of applications such as health and hygiene products, etc [5]. The antimicrobial finish obtained from the herbal extract is one of the types of special finishes, applied to the textile material. Antibacterial fabrics can be used as daily wear which reduces the effect of protecting the wearer from bacteria [6]. The textile fibers are these days increasingly treated with antimicrobial agents because of the wide application of textile materials used in medical and the pharmaceutical industry in forms of surgical gowns, surgical mask, bed spreads, pillow covers etc. this finish has also importance in sport textiles and home textiles also.

The antimicrobial finishing process imparts the ability, to textile substrate, to inhibit the growth (-or to kill at least some types of microorganisms. Therefore, an antimicrobial finish should be capable to kill the microbes by breaching the cell wall or alter cell membrane permeability [7].

The active agent used in antimicrobial finishing should be effective only against undesirable organisms. It should also be nontoxic, safe and biodegradable and should have long durability. The natural agents with their effective antimicrobial activity and less adverse consequences are being used at present to overcome the problems caused by synthetic agents. India has a rich biodiversity with more than 450 plants available for dye extraction and antimicrobial finishing [8]. Plant extracts provided an attractive source of eco- friendly antimicrobial finish [9]. Now again the interest of scientists and academicians has shifted towards the use of plants sources to develop product using natural ingredients which are of great importance due to medicinal properties. Antimicrobial screening of plant extracts and phytochemicals represents a starting point for antimicrobial finish based medical textiles [10]. The present study was aimed to determine the potential antibacterial activities of plants extracts from ten selected plants sources belonging to different families on human pathogenic bacteria. All of plants assayed in this study are commonly found in different localities of India and the renewable parts of the plants i.e. leaves are used.

Materials and Methods

Preparation of cotton fabric

The pure cotton fabric was purchased from local Hisar city of Haryana. Desizing and scouring treatments were given to the woven cotton fabric to remove foreign materials before imparting finish.

The cotton fabric was given desizing treatment using 2 mL/L Americos Amylase 543 at 60 °C temperature for 60 minutes with 1:20 material to liquor ratio by maintaining 7 pH. The treatment liquor was drained out and given one hot rinsed and cold wash and dried.

Desized cotton fabric was scoured in a bath containing 1.5% (owf) Palkoscour APCL enzyme, at 60 °C for 60 minutes at material to liquor ratio 1:15 maintained at 7.0 pH. The fabric was rinsed in hot and cold water and dried.

Collection and selection of plant material

On basis of available review, an exhaustive list of 50 plants having antimicrobial property was prepared. Out of the prepared list, ten plants having good bacterial resistance, locally available and in abundance were selected for the study. Only renewable parts of the plants were used. The fresh leaves were collected, washed to remove debris and dried at 40±1 ºC in hot air oven.

After being completely dried, the material was crushed into small pieces, pulverized into coarse powder and stored in air tight containers free from environmental climatic changes, till usage.

Table 1: List of selected plant sources

Sr. No.	Plants name (local name)	Plants (botanical name)	Plant parts used	Dye powder
1.	*Sagun/ Sagwan/Teak*	*Tectona grandis*	Leaves 	
2	*Mehandi*	*Lawsonia inermis*	Leaves 	
3	*Curry patta*	*Murraya koenigii*	Leaves 	
4	*Aonla*	*Phyllanthus emblica*	Leaves 	
5	*Bael*	*Aegle marmelos*	Leaves 	
6	*Neem*	*Azadirachta indica*	Leaves 	

7	*Nimboo*	*Citrus limon*	**Leaves** 	
8	*Safeda*	*Eucalyptus globulus labill*	**Leaves** 	
9.	*Jamun*	*Syzygium cumini*	**Leaves** 	
10.	*Peepal*	*Ficus riliigosa*	**Leaves** 	

Preparation of extract

Extraction refers to separating the desired extract by physical and chemical means with aid of solvent. Two different mediums of extraction were used. The extraction of plant materials was done using following methods:

Aqueous extract was prepared by soaking 2g of plant powder in 50 mL distilled water in a stainless steel vessel overnight to loosen the cell structure. The mixture was centrifuged and filtered to separate the extract and remove plant remnants**.**

2g of plant powder was soaked in 50 mL of 70% ethanol in a stainless steel vessel overnight to loosen the cell structure. The mixture was centrifuged and filtered to separate the extract and remove plant remnants**.**

Yield count

Aqueous extract was prepared by soaking 2g of plant powder in 50 mL distilled water in a stainless steel vessel overnight to loosen the cell structure.

The mixture was centrifuged and filtered by using Whatmann No.1 filter paper to separate the extract and remove plant remnants**.** After separating plant, the amount of extract was measured in mL.

Determination of Total Phenolic Content (TPC)

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure. Samples (2mL, triplicates) were introduced into test tubes; 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were shaked well to mix the contents and allowed to stand for 30 min.

Absorption at 765 nm was measured using Systronics UV-vis spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.[12]

Bio-Assay

The effect of various plant extracts on the two bacterial strains i.e. *Escherichia coli* and *Staphylococcus aureus* was assayed by Agar well diffusion method and antifungal activity was assessed against *Aspergillus niger*. The minimum concentrations of the plant extracts to inhibit the microorganisms were also determined by dilution method using plant fractions serially diluted in sterile nutrient broth.

Antibacterial testing of extracts

The antibacterial activity of samples 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 MacFarland scale 0.5 (10^6 colony forming unit (CFU) per mL). Petri plates containing 20 mL of Nutrient Agar was used for antibacterial activity. The inoculums was prepared on the surface of the solidified media and Whatman 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 ^{0}C. The diameters of zone of inhibition were measured in millimeters (zoi mm) [13].

Anti-Fungal efficacy of extracts

The suspension of *Aspergillus niger* which was grown for 48 h in PDA medium was made by 10^{-2} of dilution method. The suspension then inoculated into Petri dish with PDA medium and a piece of extract treated cotton sample of 10 mm diameter was placed over it. Incubation was done for 48-72 h at 37 ^{o}C in laminar flow with UV radiation**.**[14]

Results

Yield of plant materials in aqueous and ethanol extraction mediums

Table 2 shows the yield obtained from the aqueous and ethanolic extraction of plant material. The extract yield varied from 40.00 to 46 mL/50 mL in aqueous extracts. *Jamun* leaves exhibited maximum yield of 46 mL/50 mL and *nimboo, mehandi* and *neem* leaves exhibited lowest yield i.e. 45.50 mL/50 mL, 41.50 mL/50 mL and 40.00 mL/50 mL respectively in aqueous medium.

Table 2: Yield of selected plant materials in different extraction mediums

S. No.	Plant Names	Yield of extracts (mL/ 50 mL)	
		Aqueous medium	Ethanol medium
1.	*Aonla*	45.00	45.50
2.	*Bael*	44.00	44.00
3.	*Curry patta*	45.00	45.00
4.	*Safeda*	45.00	44.00
5.	*Mehandi*	41.50	43.00
6.	*Jamun*	46.00	44.00
7.	*Neem*	40.00	44.00
8.	*Nimboo*	45.50	45.00
9.	*Peepal*	45.00	45.00
10.	*Sagun/ Sagwan*	41.50	44.00

In case of ethanolic medium of extraction, yield of extract ranged from 43.00 to 45.50 mL/50 mL. The maximum yield was noticed for *aonla* leaves i.e. 45.50 mL/50 mL and the lowest yield was found in *mehandi* leaves (43 mL/50 mL). It was assessed that the yield count also varies according to the extraction medium.

Total phenolic content of plant extracts in different mediums of extraction

The data presented in Table 3 indicate that in the ethanolic extraction medium maximum phenolic content was also found in *neem* leaves (482.01 mg GAE/g) followed by *safeda* leaves (286.04 mg GAE/g**)**, *aonla* leaves (86.42 mg GAE/g), *sagun* leaves (55.15 mg GAE/g), *nimboo* leaves (42.06 mg GAE/g), *jamun* leaves (36.18 mg GAE/g), curry patta leaves (22.48 mg GAE/g), *peepal* leaves (15.66 mg GAE/g), *mehandi* leaves (11.17 mg GAE/g) and *bael* leaves (11.14 mg GAE/g).

Table 3: Total phenolic content of selected plant extracts

S. No.	Plants extracts	TPC in extracts (mg GAE/g)*	
		Ethanol extract	Aqueous extract
1.	*Aonla*	86.42	31.58
2.	*Bael*	11.14	04.21
3.	*Curry patta*	22.48	06.44
4.	*Safeda*	286.04	117.51
5.	*Mehandi*	11.17	01.59
6.	*Jamun*	36.18	10.74
7.	*Neem*	482.01	167.24
8.	*Nimboo*	42.06	23.71
9.	*Peepal*	15.66	02.65
10.	*Sagun/ Sagwan*	55.15	13.17

In aqueous medium of extraction maximum phenolic content was also exhibited by *neem* leaves (167.24 mg GAE/g) followed by *safeda* leaves (117.51mg GAE/g), *aonla* leaves (31.58 mg GAE/g), *nimboo* leaves (23.71 mg GAE/g), *sagun* leaves (13.17 mg GAE/g), *jamun* leaves (10.74 mg GAE/g), curry leaves (6.44 mg GAE/g), *bael* leaves (4.21 mg GAE/g), *peepal* leaves (2.65 mg GAE/g) and *mehandi* leaves (1.59 mg GAE/g). In both the extraction medium *neem* leaves showed the highest total phenolic content. The result of the present study indicated that the ethanol extraction medium exhibited the highest amount of phenolic compounds as compared to aqueous extract.

Bio assay

Natural extracts were screened for their antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* bacteria *and Aspergillus niger* fungus by agar well diffusion method and the zone of inhibition was measured. The results of screening test of plant sources extracted in ethanolic medium are reported in Table 4. Among the different plant extracts, the *safeda* leaves showed the higher zone of inhibition i.e. 23 and 20 mm against *S. aureus (gram +)* and *E. coli* (gram -) bacteria, respectively followed by *bael* leaves (20 mm each), curry *patta* leaves (20 and 18 mm), *aonla* leaves and *neem* leaves exhibited same zone of inhibition i.e. 18 and 16 mm, respectively. The lowest zone of inhibition was shown by *nimboo* leaves extract which was 12 mm for both gram positive and gram negative bacteria.

Table 4: Antimicrobial activity of selected plants extracted in ethanolic medium

S. No.	Plant extracts	Zone of inhibition (mm)		
		Antimicrobial activity		Antifungal activity
		S. aureus (gram +)	*E. coli* (gram -)	*Aspergillus niger*
1.	*Aonla*	18	16	07
2.	*Bael*	20	20	09
3.	*Curry patta*	20	18	06
4.	*Safeda*	23	20	10
5.	*Mehandi*	17	17	08
6.	*Jamun*	16	15	04
7.	*Neem*	18	16	11
8.	*Nimboo*	12	12	05
9.	*Peepal*	14	12	09
10.	*Sagun/ Sagwan*	13	11	07

Anti bacterial activity <6mm: Week; 7-12 mm: Moderate; >12mm: Strong

The anti fungal activity of selected plants extracted in ethanolic medium of extraction to *aspergillus niger* indicated that highest zone of inhibition was exhibited by *neem* leaves extract (11mm) followed by *safeda* (10 mm) and *peepal* and *bael* leaves (09 mm each). The lowest zone of inhibition was exhibited by *jamun* leaves (04 mm).

Table 5: Antimicrobial activity of selected plants extracted in aqueous medium

S. No.	Plant Names	Zone of inhibition (mm)		
		Antimicrobial activity		Antifungal activity
		S. aureus (gram +)	*E. coli* (gram -)	*Aspergillus niger*
1.	*Aonla*	12	11	04
2.	*Bael*	16	12	06
3.	*Curry patta*	12	07	02
4.	*Safeda*	16	09	05
5.	*Mehandi*	06	10	04
6.	*Jamun*	03	06	02
7.	*Neem*	04	06	04
8.	*Nimboo*	06	07	02
9.	*Peepal*	05	03	02
10.	*Sagun/ Sagwan*	08	04	03

Anti bacterial activity <6mm: Week; 7-12 mm: Moderate; >12mm: Strong

The results of screening test for antibacterial and antifungal activity of plant sources extracted in aqueous medium are reported in Table 5. Among the different plant extracts the *bael* leaves showed the higher zone of inhibition i.e. 16 and 12 mm against *S. aureus* (*gram* +) and *E. coli* (gram -) bacteria, respectively followed by *aonla* leaves (12 and 11mm), *safeda* leaves (16 and 09 mm).

The anti fungal activity of selected plants extracted in aqueous medium of extraction to *aspergillus niger* indicated that highest zone of inhibition was exhibited by *bael* leaves extract (06 mm) followed by *safeda* leaves (05 mm), *aonla*, *mehandi* and *neem* leaves (04 mm each). The lowest zone of inhibition was exhibited by and curry patta, *peepal*, and *nimboo* leaves (02 mm each).

Conclusion

We studied the antibacterial and antifungal properties of different plant sources for their application on cotton fabric to impart functional properties. Among the different plant extracts the *bael* leaves showed the higher zone of inhibition in aqueous medium i.e. 16 and 12 mm against *S. aureus (gram +)* and *E. coli* (gram -) bacteria and showed highest zone of inhibition i.e. 06 mm against *Aspergillus niger fungus.* But in case of etanolic medium of extraction, the *safeda* leaves showed the highest zone of inhibition i.e. 23 and 20 mm against *S. aureus (gram +)* and *E. coli* (gram -) bacteria, respectively followed by *bael* leaves (20 mm each) where as neem leaves showed the highest zone of inhibition i.e.11 mm against *Aspergillus niger fungus* in ethanolic medium of extraction. The use of these plant extract will be efficient to impart antimicrobial finish treatment on different fabrics and treated fabrics can also be utilized in field of medical textile.

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