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Finishing of cotton fabric with *Psidium guajava* herbal extract and testing its antimicrobial activity

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Abstract

Psidium guajava is commonly called *Guava*. It is spread throughout India. The tree has an enormous fiscal value since most of the parts like bark, leaves, seed and fruits are used as alternative medicine to treat a variety of diseases especially diabetes. In the present review, we try to give the information on phytochemical constituents and anti bacterial activity of extract obtained by *Psidium guajava* is given. In this study, both aqueous and ethanolic extracts of *Psidium guajava* were prepared using standard procedure. For antibacterial testing, bacteria *Staphylococcus aureus* and *Escherichia coli* were used. Disc diffusion and Minimum Inhibitory Concentration (MIC) studies were carried out to assess the antibacterial effect. *Psidium guajava* zones of inhibition of ethanolic extracts were in the range of 7.83 ± 0.76 mm and 8.67 ± 1.04 mm for *Staphylococcus aureus* and *Escherichia coli* respectively.

Keywords: Antimicrobial, physical properties, herbal, extracts, microorganisms, phytochemicals, environment friendly

Introduction

Medicinal plants have a key role in combating human health issues since the Stone Age. They act as restorative, defensive and supportive agents for human body. The World Health Organization (WHO) reports revealed that 80% of population in Asian and African countries rely on traditional medicines for primary health care necessities Kim *et al.* (2012) ^[10]. A pivotal role of plants in the health scenario is attributed to bioactive compounds, which could delay or inhibit the inception of degenerative diseases and increase life expectancy Jagadish *et al.* (2009) ^[11]. Approximately eighty percent of people in developing countries still rely on traditional medicines for their primary health care. This usually involves the use of plant extracts. Medicinal plants usually constitute an important source of new and biologically active compounds. There is a vast resource of natural antimicrobial agents, which can be used for imparting useful antimicrobial property to textiles. There are major challenges in application of natural products for textiles, as plant composition varies according to geographical location, age and method of extraction. The bulk availability, extraction, purification are other challenges in their application. Natural sources are still promising and needs more research for standardization. Standardization and scientifically approved procedure of extraction and application will promote eco-friendly garments as a whole and counterbalance the harmful effects of the present day chemicals.

Herbal textile is finished entirely with herbal extractions, without using any sort of chemicals. These herbs are applied directly to the fabric with the help of natural ingredients, so that the medicinal value of the herbs can be kept intact. No chemical process is adopted while finishing process. Ecofriendly treated cloth has the ability to protect from mosquitos, various skin diseases and other environmental harmful substrates. Since the herbal finished clothes or garments come in prolonged contact with the body. The medical properties of herbs are known to cause no damage to the body. There are many herbal products which show multi- functional finishing properties. The screening of plant extracts and plant products for multi-functional activity has shown that plants represent a potential source of new anti-infective agent. As a result, the number of bio-functional textiles with multi-functional activity has increased considerably over the last few years. Some of the herbal compounds obtained from plants

are well known for their multi-functional activity. These natural products are abundantly available in nature and are widely distributed. These plant products has multi-functional properties i.e. antimicrobial, anti-rashes, anti -septic, and mosquito repellent properties.

Guava Psidium guajava is a small medicinal tree. It is popularly known as guava (family *Myrtaceae*) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two most common varieties of guava: the red (*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *Pyrifera*). Guava is used by the food industry to produce candies, juices, jams and frozen pulp. As result of the fruit process there is a discard of the leaves, seeds, part of the peel and pulp fraction not separated in the physical depulping process. The high cost of pharmaceutical medications conduces to the search for alternative medicines to treat many ailments. In view of this, studies are necessary to confirm the effects of medicinal plants. The aim of this study is Phytochemical analysis and antimicrobial finishing of cotton fabric with *Guava Psidium guajava* herbal extract and testing against Gram positive and Gram negative bacteria.



Material and Methods

Fabric- Bleached cotton fabric washed with distil water.

Chemicals: Citric acid, Methanol, Potato dextrose Agar Guava leaves

Equipments: Autoclave, glass wares, volume pipette, sprit burners, forceps etc.

Collection of Plant material and Preparation of extract:

Fresh, mature leaves of *Psidium guajava* plant were collected from CCAS, MPUAT, and Badgav KVK, Udaipur during according to their Cultivation. Fresh leaves of *Psidium guajava* were rinsed with distilled water to remove dust and any particulate matter. Leaves were spread separately on paper sheets in a well-ventilated room. Dried leaves were ground with the help of food processor into fine powder. The powder was sieved through seiver (0.25 mm). Sieved powdered material was stored in tightly packed glass jars.

Extraction refers to the separation of the desired material by physical or chemical means with acid as solvent. The choice of solvent, temperature and time determine the efficiency of extraction. The extraction of plant materials was done using following methods:

1. Aqueous extraction: Aqueous extract was prepared by soaking 5gm of plant powder in 125 ml distilled water for overnight to loosen the cell structure. The mixture was centrifuged at 3000 RPM for 30 minute and the supernatant was filtered through Whatman no.1 filter paper to separate the extract and remove plant residue.

2. Ethanol extraction: Organic solvent extraction methods are suitable to verify antibacterial activity and sensitivity of microorganisms against human pathogens including bacteria, fungi or virus, as described by Elastal *et al.* (2005) [2]. 5gm of plant powder was soaked in 125 ml of 70 % ethanol (Distilled water 30%+ Ethanol 70%) for overnight to loosen the cell structure. The mixture was centrifuged at 3000RPM for 30 minute and the supernatant was filtered through Whatman no.1 filter paper to separate the extract and remove plant residue.

The dried powder was later stored at 4°C in air tight bottles for further studies as pointed out by Sofowora (1982) [8].

Test Micro-organisms: The pure cultures of bacteria were obtained from Department of Molecular Biology and Biotechnology, RCA, MPUAT, Udaipur.

Minimum inhibitory concentration (MIC): A minimum inhibitory concentration (MICs) is considered the good standard for determining the susceptibility of organism to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. The MIC is defined as the lowest concentration of an agent that will inhibit the visible growth of an organism after overnight incubation.

Physical / Mechanical / Wash Testing of Treated Fabric

- Fabric Weight (ASTM D 3776)
- Fabric Count (ASTM D 3775)
- Fabric Thickness (ASTM D 1777)

Tests to Evaluate Mechanical Properties: The mechanical property tests include tensile strength, elongation and abrasion resistance. The pad dry antimicrobial finished fabric samples were tested to find out the effectiveness of finish on the fabric.

Wash Testing of Treated Fabric: Evaluation of the durability of finish was carried out by dipping treated fabric in 5 percent of mild detergent (Ezee/Genteel etc.) solution for 30 minutes: keeping the ML ratio at 1: 50, followed by rinsing with cold water and drying. After that samples were washed with plain water, squeezed and dried in shade. After drying the treated fabric sample were evaluated against gram positive and gram negative bacteria.

Results and Discussion

Phytochemical analysis of plant sources: The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Prepared ethnolic and distilled water extracts of Guava leaves was subjected to phytochemical analysis to find the presence of the following secondary metabolites such as flavonoids, alkaloids, tannins, saponin and phenols.

Table 1: Phytochemical analysis of *Psidium guajava* herbal extract

Medium of extraction	Alkaloids	Flavonoids	Saponins	Phenol	Tannins	Terpenoids
Ethanol	+	+	-	+	+	+
Water	+	+	-	+	+	+

The results of the phytochemical screening, indicated the presence of Alkaloids, tannins, terpenoids, phenols etc. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Plant sources with flavonoids are good relief from different body allergies and stomach related problems. Presence of tannins is considered as antiviral, antibacterial and anti-tumour activities. These properties are very important in imparting antimicrobial property to the textiles. Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks. It can also be used as coagulant in rubber production, Mamta Saxena, *et al.*, 2013 [6].

Antimicrobial activity: Parallel streak method is a qualitative antimicrobial test used to detect diffusible bacteriostatic activity on textile materials. This method is useful for obtaining a rough estimate of antibacterial activity by the size of the zone of inhibition caused by the presence of the antibacterial agent.

- Materials required: Autoclave, laminar air flow chamber, sterile petri plates, incubator, micropipettes, inoculation loop, sterile forceps, test specimens
- Media :Nutrient agar and nutrient broth
- Method: Parallel streak method (AATCC 147)
- Test organisms : Staphylococcus aureus and Escherichia coli

Sterilized nutrient agar was poured in Petri plates and allowed to solidify firmly before inoculating. Prepare inoculum by transferring 1 ± 0.1 ml of 24 hours old broth culture needs to be transferred into 9 ± 0.1 ml sterile distilled water and mixed properly. One loopful of diluted inoculum by making five streaks of 60 mm length spacing 10 mm apart, covering the central area of the petri plates without refilling the loop. The streak lines have entire without any break in inoculation or on the media. Gently press the test specimens of size 25 mm \times 50

mm transversely across the five streaks to ensure the intimate contact with the agar surface. The plates incubated at 37 ± 2 °C for 24 hours. Examine the incubated plates for interruptions of bacterial growth along the streaks of inoculums, beneath the specimen and beyond the fabric edge (Anonymous, 2013). The average width of zone of inhibition along a streak on either side of a test specimen may be calculated using the formula:

$$W = \frac{T-D}{2}$$

Where

W= Width of clear zone of inhibition (mm)

T = Total diameter of test specimen and clear zone (mm)

D = Diameter of test specimen (mm)

Minimum Inhibitory Concentration: Preliminary screening, with aqueous and ethanolic extracts was carried out. Ethanolic extract was found effective for antimicrobial activity and it was further tested to determine the minimum inhibitory concentration (MIC) for each bacterial sample.

Stock solution: 100 ml of antimicrobial stock solution of 10 per cent concentration (10g of plant source in 100 ml of solvents) was prepared

Culture: 24 hours subculture of the test organism *S. aureus* (Gram positive) and *E. coli* (Gram negative) were serially diluted and 1×10^{-5} dilution was selected for MIC study

A separate tube containing nutrient broth (test tube 1) alone is the control. All the test tubes and control samples incubated for 24 h at 37°. After incubation the growth of the bacterial isolates in the test tubes were observed as turbidity using spectrophotometer at 600 nm. The least concentration where no turbidity was observed was determined and noted as the MIC value. Samples were tested in triplicates.

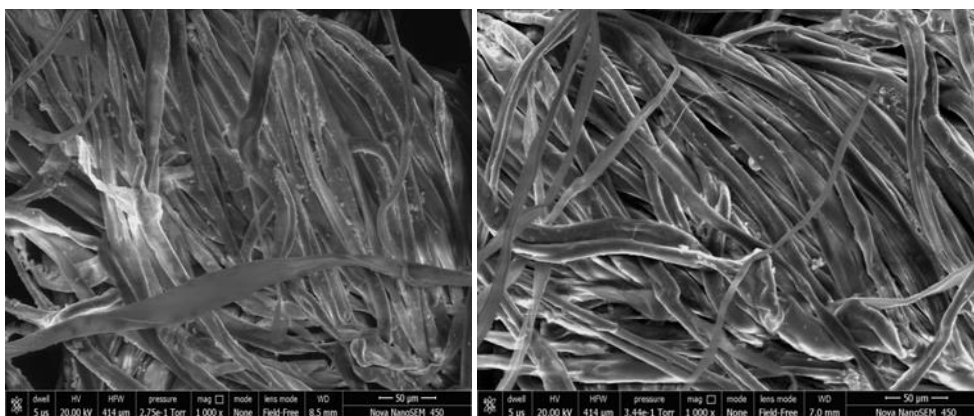
Table 2: Minimum Inhibitory Concentration of Guava Herbal extract

Test tubes	1	2	3	4	5	6	7	8	9	10	11
Volume of nutrient broth (ml)	10	9	8	7	6	5	4	3	2	1	0
Volume (V2) Antimicrobial stock solution(ml)	0	1	2	3	4	5	6	7	8	9	10
Concentration%.	0	1	2	3	4	5	6	7	8	9	10
Bacterial culture (ml) 1×10^{-5} cons.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Guava extract	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Growth is assessed after incubation for a defined period of time (24h) and MIC value is noted by visual evaluation. The complete growth of bacterial colonies was found in lower concentration it is observed that there is no bacterial growth in higher concentration. On the basis of this it can state that with increases amount of plant leaf extract it gives more clear and transparent solutions (no bacterial growth). On the basis of Minimum Inhibitory Concentration against *S. aureus* 4 per

cent concentration was selected for *Psidium guajava*.

SEM Analysis of finished fabric: Scanning Electron microscopy (SEM) of cotton fabric samples was done to analyze the effect of herbal finish imparted using plant extract on fabric. The analysis was carried out using standard methods

SEM analysis of *Psidium guajava* (L.) extract treated fabrics

Assessment of Physical Properties of treated fabric samples: Fabric weight, fabric count and fabric thickness of the pad dry finished samples were tested to find out physical changes due to amount of finish imparted on the fabric.

Table 3: Physical Properties of the *Psidium guajava* extract treated fabric

Cross linking agents	Parameters		
	Thickness (mm)	Count (no.)	GSM
Control (without)	0.326	Warp- 67 Weft- 52	0.86
Citric acid	0.339	Warp- 68 Weft- 54	0.98

Table -3 shows performance of fabric properties like fabric thickness, GSM, Count Etc. after treated with antimicrobial plant source and it is revealed that there is slightly difference in between control (which is desized and bleached fabric) and treated with cross linking agent (which is citric acid) but there is measurable difference in term of fabric GSM or fabric thickness was found. The fabric count of Citric acid treated cotton fabric showed 68 × 54 ends & picks per inch square, fabric thickness 0.339 mm., Thickness and GSM of fabric was increased after treatment with citric acid.

Prabha and Raaja (2012) [5] treated 100 percent cotton and 100 percent tencel cotton fabric using *Phyllanthus niruri* (Keelanalli) leaves and *Vetiveria zizanioides* (Vetiver roots) as natural finishing agent and citric acid as a binder. The fabric weight, thickness, count, increased in both treated fabrics.

Evaluation of Mechanical Properties: The mechanical property tests include tensile strength, elongation and abrasion resistance. The pad dry herbal antimicrobial finished fabric samples were tested to find out the effectiveness of finish on the fabric.

Table 4: Strength and elongation of the *Psidium guajava* extract treated fabric

Cross linking agents	Parameters			
	Strength (Kgf)		Elongation (%)	
	Warp	Weft	Warp	Weft
Control (without)	2.47	2.29	07.70	05.40
Citric acid	2.03	1.20	1.70	09.41

Results revealed the mean values of control and cross linking agents (citric acid) treated fabric samples. On the visual inspection, it was observed that mean value reduces in cross linking agents treated fabric as compare to control fabric, it

depicts that fabric become weaker after treated with citric acid. In term of elongation warp direction of treated fabric shows higher per cent as compare to weft direction. Results shows that fabric become slightly weaker after treated with cross linking agent (citric acid) as there is measurable difference between control and treated fabric was recorded.

Findings are confirmed with Prabha and Raaja (2012) [5] treated 100 percent cotton and 100 percent tencel cotton fabric using *Phyllanthus niruri* (Keelanalli) leaves and *Vetiveria zizanioides* (Vetiver roots) as natural finishing agent and citric acid as a binder. The tensile strength increased in both warp and weft side.

Assessment of wash durability of treated fabric samples:

The wash durability of finished fabric samples was assessed. The treated fabric samples were subjected to five, ten and fifteen wash cycles using neutral liquid soap (5 gpl) for ten minutes, keeping the material to liquor ratio at 1:30 and shade dried. The washed fabrics were later assessed for the retention of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* as per AATCC 147 standards after each subsequent wash. The antibacterial activity was assessed by measuring the zone of inhibition on either side of the test specimens and their mean and SD was taken as width of inhibition zone for corresponding concentration.

Table 5: Effect of wash cycles on antimicrobial activity of the *Psidium guajava* extract treated fabric samples against *S. aureus* and *E.coli*

Wash cycles	Zone of inhibition (mm) of treated samples	
	<i>S. aureus</i>	<i>E.coli</i>
	Mean ± SD	Mean ± SD
0 WC (Control)	7.83 ± 0.76	8.67 ± 1.04
1 WC 1	5.67 ± 1.04	7.83 ± 0.76
5 WC 5	4.83 ± 1.04	3.5 ± 1.32
10 WC	3.53 ± 0.15	3.33 ± 1.52
15 WC	2.5 ± 0.86-	2.83 ± 1.04-

Table-6 clearly depicts effect of wash cycles on antimicrobial activity of the finished fabric. The washed fabric samples were subjected to microbial testing against *S. aureus* and the bacterial growth was analyzed. *Psidium guajava* extract treated fabric samples showed presence of antimicrobial property in terms of zone of inhibition against *S. aureus* till 15 wash cycles i.e. 2.5 mm ± 0.86 *Psidium guajava* extract treated fabrics. From the results it can be concluded that *Psidium guajava* Extract treated cotton fabric showed good wash durability against *S. Aureus* The antimicrobial property decreased with increasing number of wash cycles.

Wash durability test carried out with test fabric which is treated with *Psidium guajava* leaf extracts against *E. coli* showed that the significant antimicrobial activity was actively retained in the fabric up to 15 washes even after repeated wash cycles the percentage bacterial reduction was very low and there was less activity found in the fabric after 15 washes. The effectiveness of the antimicrobial finish on washed fabric of concentration 15 also showed good antimicrobial property against *E. coli* i.e. 2.83 ± 1.04 for *Psidium guajava* leaf extracts treated fabric. From the results it can be observed that higher concentration of treated cotton fabric showed good wash durability against *E. coli* even after 15 wash cycles. The antimicrobial property decreased with increasing number of wash cycles in case of *E. coli*. and *S. Aureus*. Thilagavathi and Kumar (2005) [9] also confirmed that antimicrobial activity of neem, prickly chaff flower and pomegranate treated cotton fabric diminished gradually as the number of wash frequencies increases.

Conclusion

In the present study, the antimicrobial efficiency of Guava herbal extracts were studied. The results showed promising use of Guava herbal extracts as source of antimicrobial finishing on cotton fabric. The extract finished fabric showed maximum antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. Since leaf of *Guava* are abundantly available in Rajasthan especially in Udaipur region, the scope of implementation and commercialization of herbal extract to impart antibacterial finish in textile is high. Finally, the raw material is hundred percent from natural resources, it is ecofriendly having economic, social and environmental benefits.

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