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Bio-Synthesized Guava (Psidium Guajava L.) Leaf Treatment on Cotton Textiles as Antimicrobial Finish

KEYWORDS: Guava (Psidium Guajava L.,) leaves; Bio synthesized nanoparticles; Cotton textiles; Antimicrobial activity.

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ABSTRACT *Due to suitable climate provided by the skin, the problem of infestation was aggravated in textile materials worn, especially under garments. Bio-synthesized guava leave nanoparticles were used to treat woven and knitted cotton to impart antimicrobial (antibacterial and antifungal) property. Bio-synthesized Guava leaf nanoparticles were analyzed using UV- spectrophotometer which showed a wave-length of 423-437nm with an absorbance of 2.34 suggesting the formation of 50-200nm size nanoparticles and TEM analysis confirmed nanoparticles size with 51.8nm. 5 percent nanoparticles treatment on woven and knitted samples were treated with two methods; padding and drying at room temperature (T1) and a pad-dry-cure method at 800 C (T2) were assessed for antibacterial activity against E.coli and S.Aureus following AATCC 147-1998 and antifungal activity following AATCC 30-1993 test methods, which showed T2 treated samples have good antimicrobial activity than T1 treated samples in both type of textile materials. Even after 5-10 launderings T2 treated samples have antibacterial property, where there was no fungal growth was observed. Analysis of fabrics geometrical parameters showed an increase in yarn count, fabric count, thickness and fabric weight.*

INTRODUCTION

All natural fibers serve as a nutrient substrate for microorganisms leading themselves to damage by microbial attack. It is estimated that the total mass of all microbes living on earth is approximately 25 fold the mass of all animals. These microorganisms can be harmful to both textiles and humans, as they can cause irritation, allergy and infection with a prolonged usage of clothes near to body/ undergarments. Textiles are required to be treated with antimicrobial agents, which are non-toxic and non-allergenic. With the trend towards stringent hygienic standards and concern over environment, consumers are in search for greater protection, safety and comfort in the garments through natural sources. Recognizing the importance of plant materials as antimicrobial agents, research has been initiated in the area of producing bioactive textiles for protection of wearer from common microbes causing cross infections.

Experiments conducted in these lines showed few difficulties such as color change, stiff hand and loss in fabric strength due to the use of natural antimicrobial materials in raw form. To stabilize these properties on textiles for longer period, scientists and researchers have implicated the size and composition of plant sources by reducing into Nano-sized particles. The antimicrobial properties of guava leaf extracts and essential oils against *S. Aureus*, *Salmonella Spp* and *E.Coli* by the disc diffusion method are very active, **Goncalves *etal.* 2008**. Hence, bio-synthesized guava leaves were focused in the present study to treat cotton textiles.

METHODS AND MATERIALS:

Selection and preparation of materials

For the present research, desized woven and semi bleached knitted cotton materials were sourced from Hyderabad and Tirupur respectively, as they are most widely used materials for under garments.

Fresh good quality Guava (*Psidium Guajava* L.,) leaves were selected from the surroundings of study area, which were cleaned with ethyl alcohol and distilled water solution in 1:5 ratio; dried in tray dryer at 50^0 C; ground into fine powder; sieved and stored aseptically.

Synthesis of nano particles

Dried leaf powder was mixed in distilled water and the mixture was decanted. To this decanted solution, 1mM solution of $AgNO_3$ was added in 1:9 ratios, where the color change of broth can be observed. The broth was centrifuged at 18,000 rpm for 25 min, through which Nucleation of silver occurred.

Analysis of nano particles

UV – VIS Spectophotometer

The formation of NPs was monitored by using the UV – Visible Spectrophotometer. All the samples were measured separately. Surface Plasmon Resonance of nanoparticles was recorded. A graph of wave length on X – axis and absorbance on Y – axis was plotted.

Transmission Electron Microscopy (TEM)

Dip preparation by Flotation method was employed for TEM analysis. The sample is observed at various 25 and 30 magnifications.

Application of finish

The selected cotton materials (woven and knitted), two sets each were treated with bio-synthesized Guava leaf nano particles. 5 percent of NPs were applied through padding technique at 70 percent pressure with MLR of 1:10.

After the application one set was shade dried at room temperature (T1) and another pad-dry-cure method at 80^0C for 15min (T2). All the samples were laundered for 1, 5 and 10 times and tested for antimicrobial and geometrical properties at each stage.

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Testing of samples for antimicrobial properties
Antimicrobial property was tested through antibacterial and antifungal activity test methods as given below:

Antibacterial activity, AATCC-147, 1998
Qualitative, AATCC test method 147 was used to observe the growth inhibition of *Escherichia Coli* and *Staphylococcus Aureus*.

Antifungal acticity
AATCC 30-1993, Agar diffusion test
The growth of fungi in the conical flask was observed after 3 days.

Mycelial growth test
Prepared PD agar sterilized at 121^{0}C for 15min was poured in the petri dishes to test mycelial growth on the fabric, which was measured after 1, 3 and 5 days, **RajKumar and Krishnaveni, 2007.**

Testing fabric
Preparation of test specimen
As per BIS, the test specimens were cut to the required size using standard templates by avoiding creases and 10cms from selvedges, each were scattered as far as possible so that no two samples contained the same set of warp/weft yarns.

Atmospheric conditions
Prior to testing, the test specimens were conditioned for 24hours in the standard atmosphere of 65 $\pm$ 2 percent relative humidity and 27 $\pm$ 2^{o}C temperature in such a way to expose all portions of the material to the standard atmosphere until the moisture attains equilibrium.

Geometrical parameters
Yarn count
It defines fineness of yarn. Yarn count of fabrics was determined by the indirect method of counting the numbers of yarns per unit mass using Beesley's balance, **Booth, 1983**.

Fabric Count
Count for woven and knitted fabric is the number of warp & filling yarns and wale and course loops per unit distance (In.), respectively (ASTM, 2007). IS1963-1969, test method was used.

Fabric thickness
Thickness is the distance between one surface and it's opposite (ASTM, 2007). Heal's thickness gauge was used to measure the fabric thickness, **Booth, 1983.**

Fabric Weight
Fabric weight is used with fabrics, mass per unit area (ASTM 2007). The weight of a known size of fabric specimen was measured following IS No. 1964-1970, using a sensitive balance.

RESULTS AND DISCUSSION
Analysis of nano particles:
Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Nanoparticles analysis were determined through UV – VIS Spectophotometery and TEM.

UV – VIS Spectophotometery
From the obtained results, it was clearly evident that Guava (*Psidium Guajava* L.,) leaves was potentiality synthesized into silver (Ag) nanoparticles. During synthesis, extracted leaf broth was subjected to spectrophotometer analysis, where peak attaining a wave length of 423-437nm with an absorbance of 2.34 signifies formation of nanoparticles as shown in Figure 1. Nanoparticles exhibits dark yellowish to brown color in aqueous solution due to the surface Plasmon resonance phenomenon.

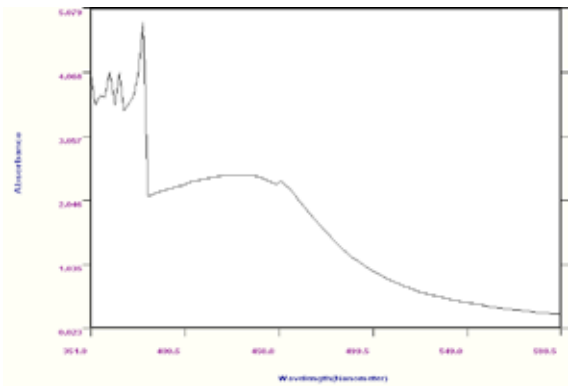

Figure 1: Wavelength of Guava leaf NPs

Transmission Electron Microscopy (TEM) analysis
The morphology of the Guava leaf nanoparticles was evaluated by TEM analysis, where TEM images at 30 magnification showed spherical shaped NPs with a dark spot at the center. This was due to the nucleation of silver with surrounded plant source, as shown in Figure 2 with a size of 51.8nm. All the nanoparticles were well dispersed within the liquid.

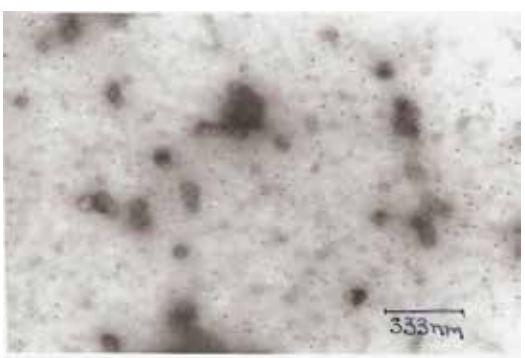

Figure 2: TEM analysis of Guava NPs

Antimicrobial analysis
Nanoparticles treated samples and untreated samples were analyzed through antibacterial and antifungal analysis.

AATCC 147-1998 – antibacterial analysis
In Agar diffusion method, the "zone of inhibition" (*zoi*) was calculated in mm, which describes the efficiency of the antibacterial property in the substrate. The *zoi* is the area around the treated substrate into which the antimicrobial chemistry leaches or moves, to kill or inhibit microorganisms, **Kavitha *etal*., 2006.**

Assessment of Nanoparticles
Nanoparticless showed *zoi* of around 6 to 8mm, where *zoi* for *S.aureus* culture showed better results than *E.coli* cultrure. This may be due to the layer of peptidoglycan in gram positive bacteria being thicker (about 20-30 nm) than gram negative bacteria.

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Assessment of antibacterial activity against *E.coli* and *S.aureus* for untreated (UT) and treated samples
as shown in Table 1, UT woven and knitted samples did not showed any *zoi* against both gram negative (*E.coli*) and gram positive *(S.Aureus)* bacterial cultures, which indicated that the UT fabric did not possesses any antibacterial activity and it can easily effected by bacteria.

The results showed that, T2 treated samples has more *zoi* than T1 treated samples, which signifies good antibacterial activity against both cultures. Among all the treated samples, knitted samples have good antibacterial activity against both cultures and effectiveness against gram positive bacteria is more than gram negative bacteria, see Table 1.

Table 1: Antibacterial activity of Biosynthesized Guava treated samples against *S.Aureus* and *E.Coli*

Treatments	Zone on inhibition*(zoi)* in mm.							
	Against *E.coli.*				Against *S.Aureus*			
	Woven		Knitted		Woven		Knitted	
	T1	T2	T1	T2	T1	T2	T1	T2
Untreated samples	-		-		-			
Treated samples	2.47	2.37	2.47	3.5	3	4	3.5	6
Samples after first wash	1	0.75	1.33	2.5	2.5	1.97	1.97	4
Samples after fifth wash	-	0.5	-	1	-	1.5	-	2
Samples after tenth wash	-	0.5	-	0.5	-	0.5	-	1

Assessment of antibacterial activity against *E.Coli* and *S.Aureus* after one, five and ten laundering
After first laundering, there was a good retention of antimicrobial activity for the samples treated with both treatment methods. Among all the samples T2 treated samples showed good activity against both cultures for both type of fabrics.

There was no antibacterial activity for T1 treated samples. It was observed that, samples laundered for more number of times had lowered antibacterial activity than the first wash and the content of nanoparticles on cotton woven and samples has decreased with an increased washings. This phenomenon is associated with the weakening of physical bonding between the nanoparticles and the cotton surface. However, even after five launderings 35-57 per cent of the antibacterial activity was retained on the samples and 21-57 per cent after ten launderings, see Table 1.

Antifungal activity
Antifungal activity was evaluated through Agar diffusion test and Mycelial growth analysis

AATCC 30-1993, Agar diffusion test
Assessment of untreated and treated samples for antifungal activity
The fungal growth was rated on four point scale analysis. After three days, untreated woven fabric showed maximum fungal grown on the top as a layer, whereas test tube with knitted fabric has developed fungus mixed within the broth, which accounts to moderate fungal growth.

T1 treated samples have shown moderate fungal growth for both type of fabrics. There was no fungal growth on T2 treated samples, and it gives good protection against fungus.

Assessment of treated samples after one, five and ten laundering for antifungal activity
No growth was observed for the T2 treatment for both woven and knitted fabrics. After first laundering no change was observed for T1 treatment, but T2 treated woven fabric have shown moderate fungal growth in the tested broth.

There was no fungal growth observed for all the treated samples after five to ten launderings. T2 treated knitted fabric have shown excellent antifungal activity at all stages.

Mycelial growth
Assessment was observed by fungal growth on first, third and fifth day.

Mycelial growth assessment of Nanoparticles
Bio synthesized NPs did not developed any fungus even at the end of fifth day.

Mycelial growth assessment on untreated and treated-samples
After 24hrs untreated woven samples has recorded 0.6mm fungus around test sample, which was further grown to three folds at the end of fifth day. Untreated knitted sample has showed fungal growth on third day, which was developed to 1mm by fifth day.

T1 treated both cotton and knitted fabrics has developed fungus only on fifth day, which signifies that the guava leaf nanoparticles can resist fungal activity upto maximum time than untreated ones. All the T2 treated samples did not shown any mycelial growth even at the end of fifth day, except knitted T2 treated sample on fifth day, which developed 0.2mm mycelial growth. T2 treatment is more intact and effective against fungal activity.

Mycelial growth assessment of treated samples after one, five to ten launderings
T2 treated woven fabric was not effected by fungus after first laundering, where after five days T1 treated sample has shown 0.3mm of mycelial growth around the test sample. T1 treated knitted fabric was effected on third with a growth of 0.1mm, which further increased to 0.4mm by fifth day. T2 treated sample has shown on fifth day with 0.2mm of fungal growth.

No fungal growth was observed after five to ten laundering, even after five days

Results of Geometrical parameters of fabric Yarn count (s)
This test was conducted for woven fabric. There was an increase in the yarn count after the samples were treated with NPs, which may be due to the shrinkage of yarns in the pre-treatment process. Compared to T1 treated samples, T2 treated samples has shown increased yarn count, this may be due to further shrinkage of yarns caused by bone drying at elevated temperature, see Table 2.

Fabric count (No. of yarns per In.)
An increased warp count was observed in treated samples over untreated ones for woven fabric. The number of wales and courses remained same for treated and untreated samples, as the knitted fabrics are compact in its structure it did not showed further shrinkage in fabric count. Though there is no great difference between T1 and T2

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treated fabrics, but there was a decreased fabric count was observed in weft direction compared to untreated samples, see Table 2.

Fabric thickness (mm)

Woven and knitted fabrics showed an average thickness of 0.282mm and 0.709 mm respectively. The increased thickness in woven samples may be due to the NPs embedded within the fabric. From Table 2, it is clearly evident that T1 treated samples are more thicker than T2 treated samples, as the yarns in T2 treated samples were flattened and protruding fibers were compressed during the padding and set during the curing process.

Fabric weight (GSM)

There was almost equal weights observed in the treated samples to untreated cotton samples. Slight increase in the weight was observed for both treatments of knitted fabrics, see Table 2.

Table 2. Geometric parameters of the test samples

Parameters		Yarn count (s)		Fabric count (No./In.)		Fabric thickness (mm)	Fabric weight (GSM)
Samples		Warp	Weft	Warp/ Course	Weft/ Wales		
Woven	UT	41.4	36.6	77	56	0.282	0.8162
	T1	42.4	38	80	53	0.306	0.8152
	T2	46	36.2	80	53	0.302	0.8008
Knitted	UT	-	-	37	36	0.709	1.68
	T1	-	-	38	37	0.72	1.79
	T2	-	-	39	35	0.682	1.77

CONCLUSION

In the present research, Guava leaf NPs are proven to be potentially a good source to finish cotton textiles for antimicrobial property. Compared to untreated samples, treated samples with both treatments (T1 and T2), were found to be a good finish against microorganisms. The results have further shown that, T2 treatment samples have good antimicrobial activity than T1 treated samples. Even though reduction in the *zoi* was observed with an increased washing, which was due to the weak bonds of nanoparticles with the fiber polymer system, the antibacterial activity lasted for 5 to 10 washes.

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