

EFFECT OF EXTRACT OF GUAVA LEAVES ON COTTON FOR MICROBIAL RESISTANCE

By

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TEXTILE AND APPAREL DESIGNING**

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CERTIFICATE-I

This is to certify that this thesis entitled “**Effect of Extract of Guava Leaves on Cotton for Microbial Resistance**” submitted for the degree of **Master of Science**, in the subject of **Textile and Apparel Designing** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Zeba Jamal, Admn. No. 2014HS22M** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of the investigation have been fully acknowledged.

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CERTIFICATE-II

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(Ms. Zeba Jamal)

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God has endowed mankind with trees and plants for survival much before his arrival on earth. They fulfilled the need of protection and modesty of humans and provided them with fruits to satisfy their hunger. Fruits are known to be an integral part of diet and are consumed fresh as juices, salads or fruit based drinks. Besides their delicious taste and flavour, the fruits are known to reduce risk of several chronic diseases including cancer. The protective nature of fruits is due to presence of phyto-constituents such as polyphenolic compounds. Guava, the most popular fruit is a powerhouse of nutrients (Madhuri, 2014).

Guava, (*Psidium guajava L.*) an important member of *myrtle* family (*Myrtaceae*) is a tropical plant found in hot climatic areas all over the world. The term Guava is also used for the fruit, which is a true berry. A berry is a fleshy or pulpy indehiscent fruit in which the entire ovary wall ripens into a relatively soft pericarp, the seeds are embedded in the common flesh of the ovary, and typically there is more than one seed. Health benefits of Guava fruit are known to most of us but we are unaware of the fact that even Guava leaves have several medicinal properties and offer an array of health benefits.

Since 1950s, Guava leaves have been the subject of diverse research initiatives to map the chemical identity of its constituents, pharmacological properties, and history in folk medicine (Gutierrez, *et al.* 2008). From preliminary medical research in laboratory settings, extracts from Guava leaves or bark are implicated in possible therapeutic mechanisms against cancer, bacterial infections, inflammation, and pain (Chen, *et al.* 2007). Essential oils from Guava leaves have shown strong anti-cancer activity *in vitro* (Manosroi, *et al.* 2006). These are also used as a remedy for diarrhoea and for their supposed antimicrobial properties (Kaljee, *et al.* 2004). Guava leaves or bark have been used traditionally to treat diabetes (Mukhtar, *et al.* 2006). Decoction of the leaves is also used as gargle for sore throats, laryngitis, external ulcers on the skin and vaginal irritations (Rouseff, *et al.* 2008). There are bioactive components in the Guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The antibacterial agent in these leaves protect the teeth and gums and therefore, Guava leaves are used as an ingredient in toothpastes and mouth fresheners. Guava leaves are also effective in eliminating acne and black spots from the skin. They contain an antiseptic that can kill acne causing bacteria. Due to these medicinal properties, Guava leaf supplements are now-a-days available in the form of capsules and Guava leaf tea (Saba, 2014).

The phenolic compounds such as α -pinene, β -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, α -bisabolene, caryophyllene oxide, β -copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, β -sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-

arabinofuranoside (avicularin) and 3-L-4 pyranoside (Essential oil), resin, tannin, eugenol, caryophyllene, azulene, Guajavolide and guavenoic acid, triterpene oleanolic acid, triterpenoids, found in Guava leaves possess antioxidant and antimicrobial properties (Kumar, 2012). Aqueous extract of guava leaves is described to be effective against a number of microbial strains.

Microbes are the tiniest creatures which cannot be seen with the naked eye and can be found almost everywhere in the environment. For the growth and multiplication of micro-organisms, the minimum nutritional requirements are water, a source of carbon, nitrogen and some inorganic salts. Micro-organisms include a variety of organisms like bacteria, fungi, algae and viruses. Bacteria grow very rapidly under warmth and moisture. Some specific types of bacteria are pathogenic and cause cross infection as these are capable of invading the body of a human, where they replicate and cause tissue damage. They can act as the carriers of some micro-organisms such as pathogenic bacteria, odour generating bacteria and fungi. Pathogenic micro-organisms like *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus spp.* and *Candidaalbicans* have been found on textiles. They damage the textile substrate and may also promote skin contamination and inflammation in sensitive people (Haug, *et al.* 2006). Microbes may also cause a decrease in fabric mechanical strength (Gao and Cranston, 2008).

Microbial action is a dominant factor limiting the service life and performance of all textiles. Textile materials are conducive to microbial attack as these provide large service area for microbial growth (Cardamone, 2002). Natural fibres have cellulose and protein which provide basic requirements for bacterial growth and their multiplication (Singh, *et al.* 2005). Cotton covers almost all the possible fields of human living from formal to informal clothing. Cotton fabrics are generally worn next to skin hence direct contact of textiles with human body provides warmth, humidity and nutrients; an excellent environment for micro-organism growth. These micro-organisms create unpleasant odour, discoloration, staining, degradation of textile materials and spread of diseases.

Bacterial growth on the textiles can be inhibited by applying chemical and natural botanical anti-microbial agents. These may kill the micro-organisms by damaging the cell wall (Madigan, *et al.* 2006), destroy the growth of micro-organisms and their negative effects of odour, staining and deterioration. In recent years, anti-microbial agents like triclosan, quaternary ammonium compounds, metal salts solutions, antibiotics and nanosilver are available for use on textiles (Mahesh, *et al.* 2011). Synthetic anti-microbial agents leads to fabric strength loss, make the fabric stiff, change the colour of the fabric and also harm the environment (Hussain, 2006). Due to these problems natural products in textile substrate are gaining significance momentum.

Natural products are a source of new chemical diversity and are the choice of today's world. The sources of natural product are plants, animals and microorganisms. Among them,

plants and plant products are more reliable for its renewability and therefore considered as catalyst for human welfare. They are the primarily required materials for health care system in some parts of the world (Mahesh, *et al.* 2011). Plants have their own self defense mechanism and protect themselves from microbes due to the presence of substances known as phytochemicals. These phytochemicals are divided into primary and secondary metabolite. Primary metabolites are the compounds involved in the metabolic pathway, which are common to all living organisms (Dewick, 2009). Secondary metabolites such as flavonoids and tannins are responsible for protection from microbes and thus exhibit potential to be used for the development of protective textiles by using different textile finishing techniques.

Many plant extracts from roots, stem, leaves, flowers, fruits and seeds of diverse species of plants exhibit anti-bacterial properties. These can be used for treating the textiles and the relatively lower incidence of the adverse reaction of these treatments coupled with reduced cost can be exploited as an effective eco-friendly alternative to the synthetic antimicrobial agents for textile applications. Herbal treatments also improve some physical properties of the fabric like non-wettability, resistance to abrasion and microbial resistance. Application of plant extract containing active substances to make the cloth microbial resistant and to safeguard the fabrics from damage by synthetic antimicrobial agents is an innovative approach.

Considerable attempts have been made to study the use of extracts of plants and trees for medicines and natural biodegradable pesticides but little information is available for the assessment of resistance of extracts of fruit tree for microbial growth on natural fabrics. Antimicrobial textiles can improve serviceability of textile products. Serviceability of textiles is the first requisite for the consumers who are increasingly becoming aware of the hygienic life and health. Hygienic textiles and congenial micro environment are prime requirements for human beings to live comfortably and healthy. Therefore, it is of prime importance to obtain information about the resistance of some herbal treatments on natural fabrics at household level to help consumers. The clothes can be treated with such plant extracts after every wash as renewable treatment. Guava leaves are available in abundance especially in autumn and its aqueous extract is described to be effective against a number of microbes like *Aeromonashydrophila*, *Shigella spp.*, *Vibrio spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus spp.* (Abreu, *et al.* 2006). Considering the health problems faced by the consumers due to environmental hazards like microbes as well as understanding the importance of cotton in our life, the present study was conducted for the application of Guava leaves extract treatment on cotton fabric for microbial resistance with following objectives:

1. To prepare the extract from leaves of Guava tree for application on cotton fabric.
2. To determine microbial resistance of the treated fabric
3. To study the efficacy of treatment on physical properties of the fabric

The main function of citing review of literature is to provide base for developing a frame work, provide insight into the methodology and suggest operational definition of the concepts and finally to work out a basis for interpretation of findings. Keeping in view the objectives of the study, the review of literature of relevance to the present study is arranged in this chapter under the following sub headings:

- 2.1 Anti-microbial properties of *Psidium guajava* (L.)
- 2.2 Taxonomy and scientific importance of *Psidium guajava* (L.)
- 2.3 Extraction techniques for Guava leaf extract
- 2.4 Application and assessment of anti-microbial activity on fabrics
- 2.5 Effect of treatment on physical properties and wash durability of treated fabric.

2.1 Anti-microbial properties of *Psidium guajava* (L.)

Sapkota *et al.* (2012) tested Guava leaves with mid ribs and without midribs against *B.subtilis*, *E.coli*, *P.aeruginosa*, *S.abony* and suggested that the inhibition zone surrounding the wells varied from 15.23 – 20.52 mm with all bacteria being sensitive to Guava leaves extract.

Biswas *et al.* (2013) conducted a study to screen the antimicrobial activity of n-hexane, methanol and ethanol and water leaf extracts of *Psidium guajava*. The extracts were screened against two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) by using agar well diffusion method. Findings revealed that only gram-positive bacteria were susceptible to methanol and ethanol extracts, while neither of the gram-negative bacterium showed inhibition. The resistance of the gram-negative bacteria could be attributed to its cell wall structure, which have an effective permeability barrier comprised of a thin lipopolysaccharide exterior membrane, which could resist the penetration of the extruding plant extract.

Garode and Waghode (2014) studied the antibacterial activity of the Guava leaves extract against *S. mutans*. The antibacterial activity of ethanol extract of Guava leaves showed optimum results against *S. mutans* with zone of inhibition 18 mm. The chloroform and benzene extract did not show any activity. While the acetone, methanol and petroleum ether extracts of Guava leaves shown moderate activity against *S. mutans* with zone of inhibition 12 mm, 11 mm and 12 mm, respectively.

Taura *et al.* (2014) extracted dried leaves of *Psidium guajava* using ethanol as extraction solvent via percolation and soxhlet method at temperature of 45 and 60°C. The leaf extracts were subjected to phytochemical screening in order to detect the presence of

secondary metabolites. The extracts were further tested for antibacterial activity against clinical isolates of *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using disc diffusion and broth dilution techniques. The results of phytochemical screening indicated the presence of alkaloids, tannins, reducing sugars, saponins and glycosides in all the three extracts. The results of antibacterial activity indicated that only *Salmonella spp.* and *Pseudomonas aeruginosa* were sensitive to all the extracts.

2.2 Taxonomy and scientific importance of *Psidium guajava* (L.)

2.2.1 Taxonomy of *Psidium guajava* (L.) (Shruthi, *et al.* 2013)

Common name: Guava

Botanical name: *Psidium guajava* (L.)

Kingdom : Plantae

Order : Myrtales

Family : Myrtaceae

Subfamily : Myrtoideae

Genus : *Psidium*

Species : *Guajava*

Binomial name : *Psidium guajava* Linn.

2.2.2 Scientific importance of *Psidium guajava* (L.)

Rahim *et al.* (2010) reported that crude aqueous mixture and water soluble methanol extract from leaf and bark of *Psidium guajava*, a tropical fruit Guava of the family *Myrtaceae*, showed strong antibacterial activity against multidrug-resistant *V. cholerae* O1. The in vitro minimum inhibitory concentration of the crude aqueous mixture and water soluble methanol extract, which was bactericidal against 10⁷ CFU/ml of *V. cholerae* was determined to be 1,250 mg/ml and 850 mg/ml, respectively. The antibacterial activity of *P. guajava* was stable at 100°C for 15–20 min, suggesting nonprotein nature of the active component. The growth of *V. cholerae* in rice Oral Rehydration Saline (ORS) was completely inhibited when 10 mg/ml (wt/vol) of crude aqueous mixture was premixed with the ORS in a ratio of 1:7 (vol. extract/vol. ORS).

Porwal *et al.* (2013) discussed extraction processes of Guava leaves with different solvent such as ethanol, methanol, ethyl acetate and water. They reached to a conclusion that aqueous extract showed its wide use in medical as compare to other solvents extract. Aqueous extract of Guava leaf have antiglycation activity and prevent neurodegenerative and cardiovascular disease and also shows antiprostata cancer activity.

Saba (2014) reported that Guava leaves are packed with antioxidants, antibacterial, anti-inflammatory agents and beneficial tannins and thus fresh Guava leaves are considered as a natural pain reliever. Guava leaves are known to contain polyphenols, carotenoids,

flavonoids and tannins. These are effective in eliminating acne and black spots from the skin. They contain an antiseptic that can kill acne causing bacteria.

These aid in weight loss by preventing the complex starches from being converted into sugars. Guava leaf tea can effectively lower blood glucose in diabetics by reducing the alpha-glucosidase enzyme activity and are herbal remedy for diarrhoea and dysentery. It can also help in curing ear infections. The powerful antibacterial agents kill bacteria in the lining of the gut and stop proliferation of toxic enzymes by bacteria. These are particularly beneficial in case of food poisoning as well as soothing, vomiting and nausea. Fresh Guava leaves can relieve tooth ache, heal gum and mouth sores and treat sore throats when used for gargling. Guava leaves are considered a natural remedy for dengue fever. This is because guava leaf extract can increase the number of platelets in the blood and is not toxic at all. Guava leaves can be beneficial in case of prostate cancer and enlargement. The antibacterial agents prevent infection and reduce inflammation of the uterus to accelerate the healing of wounds. Guava leaves contain antioxidants which destroy the free radicals that damage the skin, thus protecting it from ageing as well as improving skin tone and texture.

2.3 Extraction technique for Guava leaves extract

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts (Handa, 2008). The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction, and phytonic extraction.

Maceration extraction is crude extraction; solvents diffuse into solid plant material and solubilize compounds with similar polarity. Effect of plant material depends on its origin, variations in the extraction technique, the time, temperature of extraction, solvent concentration and polarity, quantity, and secondary metabolite composition of an extract. Variations in extraction methods are usually found in the length of the extraction period, the solvent used pH, temperature, particle size, and the solvent-to-sample ratio (Ncube, *et al.* 2008).

Preethi *et al.* (2010) selected *Ficus religiosa*, *Leucas aspera*, *Holarrhena antidysenterica* and *Psidium guajava* for the study. They immersed and mixed samples of 10g in 100 ml of distilled water, methanol & ethanol separately and allowed to soak for 24hrs. Then the mixer was filtered through Whatmann No.4 filter paper to get pure extract. They

compared antimicrobial potential of plants according to their zone of inhibition against the several pathogenic organisms. Methanol extraction of *P. guajava* showed high antimicrobial activity against the food borne pathogens like *S. typhi*, *Pseudomonas spp* and *B. Subtilis*. The high antioxidant activity of 36g/100mg was also observed in *Psidium guajava*.

Ismail *et al.* (2012) collected and shade dried the leaves of Guava (*Psidium guajava*). These leaves were extracted with methanol by cold maceration. The solvents were evaporated under vacuum and subjected to preliminary phytochemical screening. The result suggested that methanolic extracts of leaves showed significant antibacterial activity compared with standard drug.

Pandey and Shweta (2012) collected, washed, incubated and grinded the stems, leaves and barks of *Psidium Guajava* into fine powder. They conducted maceration with 70% ethanol and 80% methanol, ethyl acetate and hot water. In comparison to all parts, the stem showed best results and the zone of inhibition obtained was 28.5 mm.

Chetia *et al.* (2014) evaluated the phytochemical constituents, antioxidant, antibacterial activity and nutrient content of young twig of *Psidium guajava* and determined total phenol and flavonoid content and anti-oxidant activity of the extracts spectrophotometrically, antimicrobial activity followed by Agar well diffusion method and nutrient content by standard laboratory methods. The analysis confirmed the presence of tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, phlobatannin, alkaloids, reducing sugars and absence of saponin and anthraquinone. Total phenol and flavonoid content, and antioxidant activity was found higher in methanol extract than ethanol extract. Positive antibacterial activity was found against *B. cereus* and *S. epidermis* in case of ethanol extracts and against *B. cereus*, *S. epidermis*, *E. coli*, *S. aureus*, *P. vulgaris*, in case of methanol extracts.

Ekundayo *et al.* (2014) weighed one hundred grams of the fine powdered leaves of the guava (*Psidium guajava*) and transferred into a clean labeled conical flask containing measured 250 ml of distilled deionised water and allowed to percolate for 24 hours. The resulting extract was filtered. The filtrate called aqueous extract of guava leaves was evaporated to dryness using thematically controlled water bath at 50-60°C. The concentrated extract after evaporation, dark brown, gummy in nature was stored in an air-tight container. The residues for the aqueous extracts were thereafter; transferred into a clean labeled conical flask containing 250 ml of 95% ethanol, shaken gently for thorough soaking and allowed to percolate for 24 hours. At a potency of 1000mg /disc, the aqueous extracts produced a diameter of zone of inhibition of 12mm against *E.coli*. and *S .aureus* respectively while the ethanolic produced diameter zone of inhibition of 12mm and 10mm against the bacteria, respectively .These results suggest that aqueous extraction of Guava leaves gave better results as compared to ethanolic extraction.

Mailoa *et al.* (2014) determined the antimicrobial activities of tannin extracts from guava leaves against pathogens microbial. The Guava leaves were extracted by maceration. They weighed as much as 50 g of guava leaves, soaked in 150 ml of ethanol with a concentration of 30%,50% and 70% for 24 hours and then filtered to obtain a filtrate. The method used for qualitative analysis with the tannins were formed by the intensity of the color is blackish green FeCl_3 compounds. In the quantitative analysis of tannins used variations of organic solvent (ethanol with a concentration of 30 %, 50 %, 70 %). Level of tannins in the sample solution was calculated with Tannates Acid Equivalent (TAE) and antimicrobial activities by using diffusion agar method. The results showed that the best solvent to extract the highest levels of tannins was with ethanol 30 % by value of tannin levels 2.351mg /g. Tannin inhibitory activity on five different pathogens was seen. The results showed that the tannins extracts can inhibit the growth of *Escherichia coli*, *Pseudomonas aureginosa*, *Staphilococcus aureus*, *Aspergillus niger* and *Candida albicans*.

2.4 Application and assessment of anti-microbial activity on fabrics

Wasif and Ruble (2007) attempted to impart antimicrobial finishing on cotton woven fabric using Aloe Vera extract at various concentrations (5, 10, 15, 20 & 25 g/l) in presence of eco-friendly cross linking agent 'glycoxal' (100g/l) by pad-dry-cure technique. Both the qualitative (AATCC-147) and quantitative (AATCC -100) evaluation was done to assess the degree of antimicrobial activity of Aloe Vera treated cotton fabric. The result showed that the number of colony counts in Aloe Vera treated cotton fabric was lesser as compared to that of untreated one.

Thilagavathi and Kannaian (2008) conducted a study on application of prickly chaff leaves (*Achyranthees aspera* Linn.) used in healthcare textiles. The pad-dry-cure method was used for application of this herbal extract on cotton fabric using citric acid as cross linking agent. The antimicrobial activity of finished fabric was assessed against gram-positive and gram-negative bacteria, by both quantitative method (AATCC-100) and qualitative methods like 'parallel streak' (AATCC-147) and 'agar diffusion' method. The finished cotton fabric showed the bacterial reduction percentage of 92 and 50 against *S. aureus* (gram- positive) and *E. coli* (gram-negative) bacteria respectively.

Mahesh *et al.* (2011) conducted a study on antimicrobial textile finish using certain natural plant products (pomegranate, *Neem* and turmeric) by keeping in mind the importance of plant based natural dyes and other bioactive natural extract in textile finishing. Plant extracts were applied on cotton by using exhaust coating and dip method. All the three plants coated on cotton showed better results against gram- negative bacteria as compared to gram-positive bacteria. Present study also proved that several plant extract could be effective against both gram-positive and gram-negative bacteria depending on the type of components present in the plant extract.

Zhang and Deng (2011) have taken extracts from Chinese herbs (scutellaria baicalensis, Chinese gall, honeysuckle and dandelion) as the antimicrobial agents, the bleached cotton fabric was treated with these extracts by padding method. The antibacterial effect of finished cotton fabric was tested by using *Bacillus coli* and *Bacillus subtilis* as the test bacteria on behalf of gram-negative and gram-positive bacteria respectively by the AATCC test method. It was reported that the finished bleached cotton fabric possesses the antibacterial effect against the gram-negative and gram-positive bacteria. When the concentration of the antibacterial agents increases, the antibacterial effect is increased. But the increase of antibacterial effect is not in proportion to the increase of the concentration of the antimicrobial agents.

Syamili *et al.* (2012) studied the antibacterial property of extract of edible Green tea leaves on cotton fabric. The extract was made with interacted with copper in copper vessels for enhancing the antibacterial properties. The extract was applied onto cotton fabric using a standard pad-dry-cure process with and without a cross linker (Citric acid). Using the standard agar diffusion method to test the antibacterial activity and durability of GTE and GTC treated cotton fabric before and after wash. After 10 washes the directly applied GTE and GTC extracts do not show much activity when compared to the GTC treated cotton with citric acid.

Saini (2014) conducted a study to assess the efficacy of *karanja* extracts on cotton fabric with respect to microbial resistance. *Karanja* extracts of leaves and seeds in viscous and dry state in two concentrations i.e. 2.5g/l and 5g/l were prepared and scoured cotton fabric was impregnated with these prepared solutions (LEVS, SEVS, SDLP and SDSP) separately. The antiseptic containing chloroxylenol treatment was taken as standard. *Pseudomonas* bacterium and *Aspergillus* fungus were selected as cotton is susceptible to their growth. The treated samples were tested for microbial (bacterium and fungus) resistance activity quantitatively by AATCC-100 test method before and after washing of samples.

Yimer *et al.* (2014) studied the antibacterial property of essential oil extracted from Eucalyptus leaves by steam distillation process using soxhlet apparatus. The extracted oil was applied on cotton fabrics by pad-dry-cure method using padding mangle. Extracted oil from these leaves showed 81.1% with acetic acid and 79.9% with citric acid resistance to antibacterial growth.

Gupta (2016) developed UV protective and antibacterial finish on cotton fabrics (woven and knitted) by using plant extracts. *Syzygium cumini* (L.) (*Jamun*) was subjected to soxhlet extraction by using methanol as solvent. The finish was applied with pad-dry-cure process by using the optimized conditions. The finished fabrics were assessed for antibacterial property against two gram positive bacteria i.e. *S. aureus* and *B. subtilis* by AATCC- 100 test method before and after washing. Ultraviolet protection factor of controlled, finished and

washed fabric was determined by AATCC-183:2004 test method. The antibacterial properties of finished cotton fabrics were 95.67% for (*Staphylococcus aureus*) for woven and 96.93% for knitted and 94.70 % for (*Bacillus subtilis*) for woven and 97.00 % for knitted, the results exhibited remarkable percent reduction in bacterial growth. The UPF value was found 'excellent' after the finishing treatment in both the finished fabrics (woven & knitted) hence showed excellent UV protective property.

2.5 Effect of treatment on physical properties and wash durability of treated fabric

2.5.1 Effect of plant extracts on physical properties of fabrics

Sathianarayanan *et al.* (2010) prepared an eco-friendly natural antibacterial finish from the plant extracts for textile application. Herbal extracts from *Ocimum sanctum* (Tulsi leaf) and rind of *Punica granatum* (Pomegranate) were applied on cotton fabric by direct application method, micro-encapsulation, resin cross-linking and their combinations. All the treatments exhibited good antibacterial properties for the fabrics. The surface morphological studies using SEM show the surface coating, microcapsules and some fibrillation. The GC-MS studies reveal that the major components responsible for the antibacterial properties are Eugenol, Germacrene and Phytol. A small decrease in tensile strength and crease recovery angle was observed for resin treated and micro-encapsulated fabrics respectively. But in the combined processes no significant changes were observed.

Ali *et al.* (2011) studied that cotton fabrics finished with Aloe Vera gel along with 1, 2, 3, 4-butanetetracarboxylic acid as a crosslinking agent using the pad-dry-cure method. The finished fabrics were characterized by Fourier transform infrared spectroscopy. The infrared spectra confirmed that the active ingredients of Aloe Vera gel attached to the hydroxyl groups of cotton fabric via a carboxylic acid cross-linking agent. The antibacterial activity of Aloe Vera-finished fabrics was qualitatively evaluated by the AATCC-147 method and scanning electron microscopy. It was observed that Aloe Vera gel-finished [concentration -3 % (w/v)] cotton fabric inhibited the growth of both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. After treatment significant changes were observed in performance properties such as tensile strength, crease recovery angle, bending length, etc.

Hein *et al.* (2013) extracted the antimicrobial agent from Aloe Vera gel with methanol and investigated the effect of antimicrobial activity of Aloe Vera gel extract on bleached cotton fabric. Qualitative analysis was carried out to measure the antimicrobial activity against gram-positive (*S. aureus*, *B. subtilis*, *B. pumilus*) and gram-negative (*Pseudo*, *Candida*, *E. coli*) bacteria. The physical properties of treated and untreated cotton fabrics such as sample width, fabric weight, fabric thickness, fabric count, yarn number, absorbency, abrasion, crease recovery, fabric stiffness and strength were analysed. The results revealed that the antimicrobial Aloe Vera gel treatment does not affect on the properties of bleached cotton fabric. The results also showed that the antimicrobial activity of Aloe Vera gel treated

fabric is excellent for *Pseudo and E-coli*, good for *B.subtilis and B.pumilus* bacteria. But, this antimicrobial agent could not inhibit the growth of *S.aureus* and *Candi*.

Saini (2014) conducted a study to assess the efficacy of *karanja* extracts on cotton fabric with respect to microbial resistance. *Karanja* extracts of leaves and seeds in viscous and dry state in two concentrations i.e. 2.5g/l and 5g/l were prepared and applied on cotton fabric. The antiseptic containing chloroxylenol treatment was taken as standard. There was significant increase in the physical properties of treated samples with increase in extracts' concentrations except air-permeability, moisture regain and tensile strength. After washing, there was significant decrease in the physical properties except air-permeability, moisture regain and elongation in all treated samples including antiseptic containing chloroxylenol (standard).

Sood *et al.* (2014) made an attempt to study the effect of functional herbal extract treatment of *Woodfordia fruticosa* on fabrics of non-woven polyester covering fabric of sanitary napkins. Physical and performance properties of the treated fabrics were evaluated to assess the effect of the herbal antibacterial treatments on these properties viz. fabric weight, fabric thickness, bulk, fabric stiffness, air permeability & wetting. There was a significant change in the performance properties of the treated polyester fabric.

Gupta (2016) developed antibacterial finish on cotton fabrics (woven and knitted) by extract of using *Syzygium cumini* (L.) leaves applied with pad-dry-cure process by using the optimized conditions. After finishing treatment in woven fabric there was decrease in fabric count, tensile strength, bending length while there was increase in weight, thickness, crease recovery angle and flexural rigidity when compared to control fabric. In knitted fabric there was decrease in stitch density, bulk, bursting strength and bending length while there was increase in weight, thickness, crease recovery angle and flexural rigidity when compared to control fabric.

2.5.2 Effect of washing on efficacy of extract treatments

Thilagavathi *et al.* (2007) imparted antimicrobial finish to the cotton fabric using extracts of *Neem* and Mexican daisy by direct application and by microencapsulation using pad-dry-cure method. To enhance the durability of antimicrobial finish to number of washes, the microencapsulation of herbal extracts has been done using phase separation/coacervation. Microcapsules are produced using herbal extracts as core and acacia as wall material. Structure of microcapsules has been evaluated using light microscopy with image analysis technique, the presence of microcapsules by scanning electron microscopy, the antimicrobial efficacy by quantitative method in terms of bacterial reduction, and the wash durability of antimicrobial activity by AATCC 124. It was observed that the microencapsulated herbal extracts possess a very good resistance for microbes even after 15 washes.

Jothi (2009) had applied Aloe gel to 100% cotton fabric to develop anti-microbial fabric. In order to optimize the process parameter, cotton fabric was treated with Aloe Vera extract (*Aloe barbadensis Mill*) at various concentrations that is 1, 2, 3 and 5 g/l at 60°C for 30 min by pad-dry-cure method. The finished fabric samples had been tested for activity as per the AATCC (Agar diffusion) method and quantitative analysis test method. The Aloe gel treated fabrics exhibited antimicrobial activity against the *Staphylococcus aureus* (AATCC 6538). The treated cotton fabrics had shown excellent antimicrobial activity at 5 g/l concentration. The wash durability of the treated sample was found good even after 50 washes.

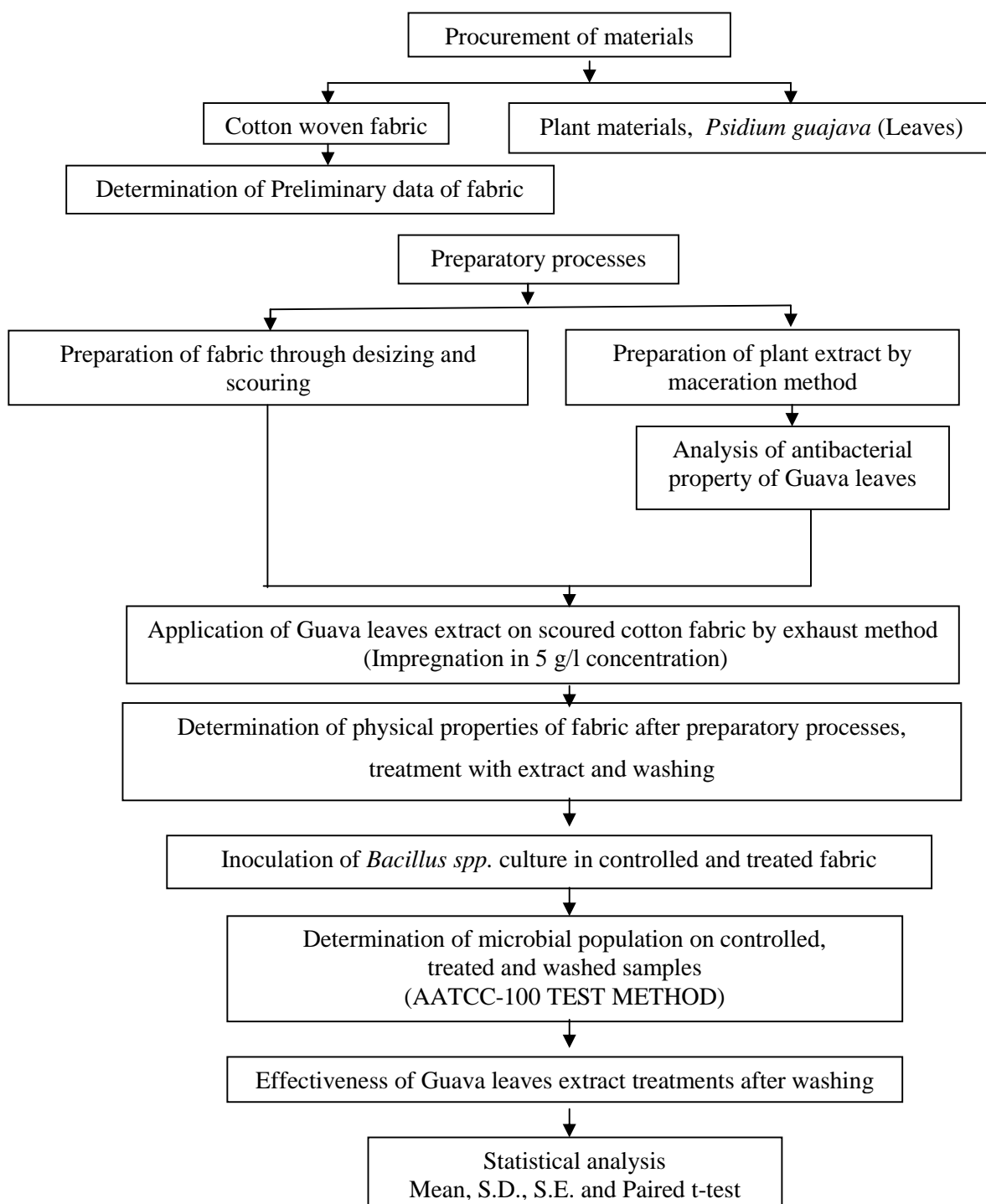
Rajendran *et al.* (2011) investigated the antibacterial performance of *Neem* with chitosan on cotton fabric. It was found that the treated fabric showed 100% exhibition against *S. aureus* bacteria. The fabric showed the good durability of antibacterial finish after 20 washing cycles. The bacterial percent reduction was 85% after 20 washing cycles. It decreased after 30 washing cycles and became 82%. There was decrease in percent reduction rate against bacteria with the increase in number of washing cycles.

Chandrasekar *et al.* (2013) finished cotton fabrics with chitosan and herbal extract nanocomposites to enhance the efficiency of biological, chemical and physical properties. *Senna auriculata* and *Achyranthes aspera* extracts mixed with chitosan solution was applied with bulk finishing and nanocomposite finishing on cotton fabrics. Antibacterial activity of nanocomposite finished fabrics showed more inhibitory zones of 30.9 mm for *E. coli* and 28.9 mm for *S. aureus* when compared to bulk finished fabrics which had a zone size of 24.9 mm for *E. coli* and 23.9 mm for *S. aureus*. Similarly Nanocomposite finished fabrics showed good durable properties and physical properties than bulk finished fabrics. The study concludes that, nanocomposites finish could provide better functional properties to the fabric than the bulk finished material. The nanosized particles in the composites were considered significant for its functional applications in hospital based fabrics to prevent the transmission of nosocomial infections.

Hooda *et al.* (2013) studied that Aloe Vera, a naturally available herb which is now increasingly being used as a functional finish on textile substrate to impart antimicrobial characteristics. Aloe Vera extract was applied by pad-dry-cure method in two concentrations (3g/l and 5g/l) on grey as well as enzymatically scoured wool fabric. Weight add-on percent of extract on treated wool fabric was determined. The antibacterial activity of the finish was accessed quantitative by AATTC- 100 test method in terms of bacterial reduction. Effectiveness of finish was assessed after 5, 10, 15 and 20 washing cycles. The results indicated that weight add-on percent was increased as the concentration of extract increased. Aloe Vera treated wool fabric showed 82.81% bacterial reduction even after 20 washing cycles. Aloe Vera treated scoured wool fabric showed very good antibacterial activity than Aloe Vera treated grey wool fabric.

Gupta (2016) developed antibacterial finish on cotton fabrics (woven and knitted) by using *Syzygium cumini* (L.) leaves. The leaves were subjected to soxhlet extraction by using methanol as solvent. The *Syzygium cumini* (L.) leaves extract exhibited moderate antibacterial properties with zone of inhibition of 11mm for *Staphylococcus aureus* and 12 mm for *Bacillus subtilis*, The application of finish was performed with pad-dry-cure process by using the standard conditions The finished woven fabric after subjecting to (0,5,10,15,20) number of wash cycles resulted in gradual decrease in antibacterial property with 95.67% to 14.19 % percent reduction in bacterial count for *Staphylococcus aureus* and 94.70% to 37.56% percent reduction in bacterial count for *Bacillus subtilis*. The finished knitted fabric after subjecting to (0,5,10,15,20)number of wash cycles resulted in gradual decrease in antibacterial property with 96.93% to 23.27 % percent reduction in bacterial count for *Staphylococcus aureus* and 97.00% to 31.22% percent reduction in bacterial count for *Bacillus subtilis*.

RESEARCH DESIGN



This chapter deals with the materials used and methods followed for conducting the present study. The different methodological procedures adopted for the study have been described and discussed under following heads and sub-heads:

- 3.1 Selection and procurement of materials.
- 3.2 Determination of preliminary data of the cotton fabric
- 3.3 Preparation of cotton woven fabric
- 3.4 Preparation of Guava leaves extracts for treatment of cotton woven fabric
- 3.5 Analysis of antibacterial property of Guava leaves extract
- 3.6 Application of Guava leaves extract on scoured cotton fabric
- 3.7 Selection, preparation and inoculation of bacterium on treated samples
- 3.8 Determination of physical properties of fabric after preparatory processes, treatment with extract and washing
- 3.9 Determination of microbial population on treated samples
- 3.10 Effectiveness of Guava leaves treatment with respect to washing
- 3.11 Statistical Analysis

3.1 Selection and procurement of materials

The cotton fabric and Guava leaves were selected as per plan of work. Two types of materials namely **cotton woven fabric** and **leaves of Guava plant** were procured.

A. Procurement of cotton woven fabric: A survey was conducted in local market of Hisar city of Haryana state to procure cotton woven fabric. On the basis of visual inspection, pure grey cotton woven fabrics suitable for apparel during summer season were collected. The collected fabrics were subjected to burning, physical and chemical tests for conformity of pure cotton fabric. The cotton fabric in grey state exhibiting light to medium weight was selected for the study. The selected cotton woven fabric was procured from Shop no. 19, Teliyan Pull Market, Hisar.

B. Procurement of leaves of Guava plant: Fresh, fleshy, mature and green leaves of Guava plant (Hisar *Surkha*) were collected from Agriculture Farm of CCS Haryana Agricultural University Hisar, because of easy accessibility and Hisar *Surkha* is a developed hybrid variety of Guava of CCS Haryana Agricultural University, Hisar.

3.2 Determination of preliminary data of the cotton fabric

Preliminary data of the selected fabric sample was done by using standard test procedure.

i) Fabric count: Fabric count is the number of warp yarns (ends) and weft yarns (picks) per inch in a woven fabric. Paramount pick glass with pointer was used to determine the fabric count using ASTM-D123 test method. It was determined by counting the number of threads per square inch in the warp and weft directions at five different places in the fabric. An average of five readings was taken as the fabric count.

ii) Fabric thickness: Fabric thickness is defined as the distance between two parallel surfaces while exerting a specified pressure on the material by pressure foot of the tester. Paramount thickness tester was used to determine the thickness of the fabric using BS2544:1967 test method. A fabric sample of 2"x2" size was placed on the flat surface under the pressure foot of the instrument, without

any fold and wrinkle. The pressure foot was lowered upon the specimen gently until the pointer of the dial meter stopped moving further and the reading on the dial gauge in mm was recorded. An average of five readings was calculated and taken as the fabric thickness.

iii) Fabric weight: Fabric weight is defined as the weight of a known area of the fabric and then computing the weight per unit area.

Fabric samples from fabric were cut at random with the help of round cutter from GSM (grams per square meter). The samples were weighed separately on the Paramount Precision Scale for GSM using ASTM-D3776-90 test method. An average of five readings was taken as the weight per unit area in grams per square meter.

3.3 Preparation of cotton woven fabric

To ensure complete wetting and uniform absorbency of the extract, cotton woven fabric was subjected to preparatory processes. Cotton woven fabric was desized in a solution containing 1 percent sulphuric acid at 50°C for 60 minutes with material to liquor ratio 1:40. The fabric was thoroughly rinsed to remove starch and acid residues. The fabric was dried and weighed. After desizing the fabric was soaked, squeezed, and treated in a solution containing 1 percent soap, 3 percent soda ash and 0.5 percent sodium sulphite at boiling temperature (100°C) with 1:40 material to liquor ratio for 60 minutes for scouring. After that the fabric was rinsed thoroughly and dried on a flat surface (Saini, 2014). Samples taken from scoured cotton fabric was kept as controlled sample.

3.4 Preparation of Guava leaves extract for treatment of cotton woven fabric

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant from the inactive or inert components by using water or selective solvents depending upon its polarity in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts (Handa, 2008).

Collected leaves were washed and allowed to dry in shade to avoid breakdown of important phytochemicals. These were crushed and grinded by grinder mixer to make into a fine powder. After that, the powder was sieved to remove the dirt and unkind particles. The dry powder was weighed and subjected to aqueous extraction for 12 and 24 hours separately. Extraction was carried out by maceration which was selected on the basis of review (Ismail, 2012). Maceration extraction is crude extraction; solvent diffuse into solid plant material and solubilize compounds with similar polarity (Ncube *et. al.*, 2008). In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing (Handa, 2008).




| Condition of Guava leaves | | |
|---|--|---|
|  |  |  |
| Fresh leaves | Dried leaves | Grinded leaves |

Plate 1 : Fresh, dried and grinded leaves of Guava tree

Maceration process: 100 gm dry powder of Guava leaves was placed in two closed vessels containing 800 ml distilled water (Yimer *et al.*, 2014) for 12 and 24 hours separately at room temperature. During this period shaking was done occasionally. After completion of time period, the liquid was strained and then filtered to make a clear liquid. This filtered solvent was subjected to rotatory evaporation at 60°-70° temperature for 2-3 hours. At last the solidified mass of the aqueous extract of Guava leaves was obtained. The extract obtained was weighed and percentage yield was calculated in terms of air dried powder weight of the plant material using the following formula.



Plate 2 : Maceration process

$$\text{Yield percentage} = \frac{\text{Yield obtained (g)}}{\text{Weight of the dry plant material (g)}} \times 100$$

The time period having greater yield percentage was selected for extraction.



Plate 3 : Guava leaves extract

3.5 Analysis of antibacterial property of Guava leaves extract

Agar well diffusion method of Dey *et al.*, 2010 with slight modification, was employed to study the antibacterial property of plant extracts. The experiments were conducted under controlled conditions in the Laminar Flow Chamber. Following steps were undertaken to test the antibacterial property of the plant extracts:

3.5.1 Preparation of broth: Mery mandel broth containing 2gm KH_2Po_4 , 1.4 gm $(\text{NH}_4)_2\text{So}_4$, 0.3 gm CaCl_2 , 0.25 gm Peptone, 0.2 gm Yeast extract, 5 mg FeSo_4 , 5.6 mg MnSo_4 , 3.34 mg ZnSo_4 , 2 mg CaCl_2 , 1ml Tween 80 were mixed with one liter of distilled water in a conical flask and stirred to get a homogeneous mixer. Solution was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The prepared medium was cooled and stored at 25°C .

3.5.2 Preparation of nutrient agar: Twenty eight gms of nutrient agar (composed of 5 gm of peptic digest of animal tissue, 5 gm sodium chloride, 1.50 gm beef extract, 1.50 yeast extract and 15 gm agar) was suspended for five minutes in one liter distilled water in a conical flask and mixed well. For complete dissolution, the solution was sterilized by autoclaving at 15lbs pressure at (121°C) for 15 minutes. The prepared mixture was cooled and poured into sterile petri plates and allowed to solidify in Laminar Flow Chamber.

3.5.3 Agar well diffusion method: The modified agar well diffusion method of Dey *et al.*, (2010) was employed to study the antibacterial property of the plant extracts. The Nutrient Agar medium (28 g nutrient agar in 1000 ml distilled water) was prepared as described in section 3.4.2. After autoclaving, the media was poured into sterilized petri plates with a uniform thickness of approximately 4 mm. The agar plates were allowed to solidify at ambient temperature and were used after 24 hours. Four uniform wells were prepared with the help of sterile cork borer of 5 mm diameter in agar plates. The entire agar surface was then inoculated with bacterial culture by spreader and left for 3 to 4 minutes. Different concentrations 1mg/ml, 3mg/ml and 5mg/ml of Guava leaves extract were added to the grooves and one control (sterilized distilled aqueous) was added to groove on agar plate (Plate 4). The plates were incubated for 24 hours at 30°C . Plates were examined and zone of inhibition of the bacteria growth was measured in mm by using diameter measurement scale. The assays were performed in triplicate and expressed as average values. Antibacterial activity of Guava leaves extract and control was interpreted as no activity (- mm), weak (<6mm), moderate (7-12mm) and strong activity (>12mm).

3.6 Application of Guava leaves extract on scoured cotton fabric

Extract was applied in one concentration (the one exhibiting the best zone of inhibition) on scoured cotton fabric by using standard exhaust method (Hooda, 2012). The concentration was selected on the basis of analysis of antibacterial property of extract.

Exhaust method is the process of application of any plant extract, chemical or dye on the surface of the fabric with the help of high temperature with any binding agent. The

method is so named because it is supposed to have absorption to the maximum limit of exhaustion.

3.6.1 Application of Guava leaves extract on cotton fabric by exhaust method: On the basis of weight of fabric samples, quantity of Guava leaves extract and citric acid as cross linking agent was calculated. The material to liquor ratio was taken as 1:20. Guava leaves extract was taken in one concentration (5g/l). This was set in a bath. Eight percent citric acid on the basis of weight of the fabric was used. The water was taken in water bath. The temperature of the water bath was raised up to boiling point. Acetic acid was used to maintain the pH of bath i.e. 5-6. Guava leaves extract was dissolved in water in a beaker. The solution was filtered. The extract was added into the water bath and stirred continuously. The samples were dipped into the bath. The bath temperature was maintained upto boil and it was kept for 30 minutes. After 30 minutes, the bath was cooled to room temperature. The samples were taken out, rinsed with lukewarm water and washed with cold water and dried. A post-treatment was given with eight percent citric acid on the basis of the weight of fabric as fixing agent. The samples were immersed in respective water bath having the fixing agent and kept at room temperature for 30 minutes which can be excluded at household level. The samples were taken out, rinsed with cold water and air dried (Hooda, 2012).

3.7 Selection, preparation and inoculation of bacterium in treated cotton fabric samples

Cellulose degrading bacterium i.e. *Bacillus spp.* was selected for the present study as cotton is susceptible to its growth and culture was also readily available in the Department of Microbiology, college of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar for use in research.

Microbial culture: Culture of procured bacterium was maintained on Carboxy Methyle Cellulose petri plates and stored at 4°C.

Composition of Carboxy Methyle Cellulose medium used is given below:

| Composition | Amount (g/l) |
|--------------------|---------------------|
| CMC | 5gm/ l |
| Glucose | 20 gm/ l |
| Yeast Extract | 5 gm/ l |
| Agar | 20 gm/ l |

The medium was prepared in distilled water. The ingredients were autoclaved at 15 lbs pressure for 20 minutes. Fresh slant culture was used for inoculation into broth. Cultures of bacterium were raised on Carboxy Methyle Cellulose medium broth at 30°C after 2 days. For preparing Carboxy Methyle Cellulose medium, all ingredients of composition were calculated and mixed properly. The volume was made to one liter for medium and agar was

added. The prepared media was poured in conical flask. Samples were taken in beaker and covered through foil. The media, controlled, treated and washed samples were autoclaved to sterilize at standard conditions (120°C; 15 lbs) for 30 minutes. The autoclaved medium and all fabric samples were inoculated. The agar plate was prepared by pouring the solid culture into sterile circular plates and allowing it to solidify in the Stericlean Vertical Laminar Flow chamber. A series of 8 test tubes containing 9 ml water were taken and sterilized through autoclave. The dishes and series of test tubes were marked for identification. Serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8}) were prepared. Only serial dilutions 10^{-7} and 10^{-8} were carried out to determine microbial counts on the basis of review (Abah and Egwari, 2011). One hundred microliters of microbial culture was uniformly transferred onto the prepared petri dish and spread with the help of spreader under laminar flow. The inoculated broth was incubated at 30°C for microbial counts on 0 day (soon after 24 hours of inoculation), 7th, 14th, 21st and 28th days. The plates were placed in an incubator for bacterium at 30°C. After 24 hours the colonies of bacterium were counted manually (Saini, 2014). The results were enumerated as percent reduction in the bacterium count of the treated samples in comparison to the controlled and treated samples and were calculated as under:

Formula used for CFU calculation :

$$\text{CFU} = \frac{\text{No. of colonies}}{\text{Amount plated (ml)}} \times \text{dilution factor} \quad (\text{Malpani, 2013})$$

Where,

CFU-Colony Forming Unit

$$\% \text{ Reduction} = \frac{\text{Initial cell no.}-\text{Final cell no.}}{\text{Initial cell no.}} \times 100 \quad (\text{Karolia, 2007})$$

3.8 Determination of physical properties of fabric after preparatory processes, treatment with extract and washing

Physical properties of treated and washed samples were tested to analyze the effect of Guava leaves extract, with respect to their controlled samples. The fabric samples were conditioned for 24 hours prior to determination of fabric properties dimensions under standard test conditions, i.e. relative humidity of $65 \pm 2\%$ and $21 \pm 1^\circ \text{C}$. All the tests were performed for three types of samples i.e. controlled (scoured), samples treated with Guava leaves extract and treated samples after subjecting to one wash cycle. The samples were subjected to tests according to the test standards as mentioned in Table 1 for respective physical property tests.

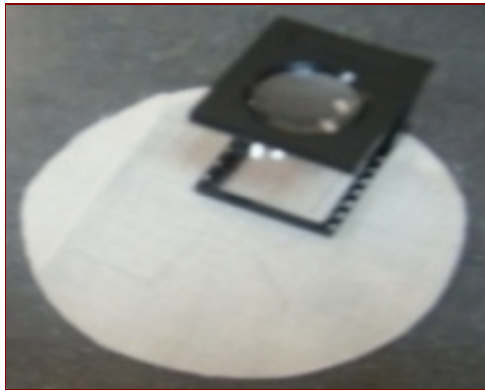


Plate 4: Paramount Pick Glass



Plate 5: Shirley Air Permeability Tester



Plate 6: GSM Quadrant Balance

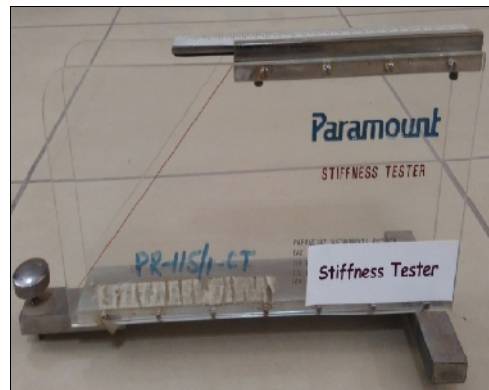


Plate 7: Paramount Stiffness Tester



Plate 8: Thickness Tester



Plate 9: Paramount Universal Tensile Tester

Table 1 : Physical properties, instruments and standards used

| Physical properties | Sample size | Instruments used | Standards used |
|--|---------------|--|----------------|
| Fabric count (ends and picks per sq. inch) | 6"× 6" | Paramount pick glass with pointer | ASTM-D123 |
| Weight per unit area (g/m ²) | Template size | GSM quadrant balance | ASTM-D3776-90 |
| Thickness(mm) | 5"× 5" | Thickness Tester | BS 2544:1967 |
| Bulk(cm ³ /gm) | 2"×2" | Electronic weighing balance and thickness tester | BS 2544:154 |
| Tensile strength (kg) | 12" × 2.5" | Paramount universal tensile tester | ASTM-D1682-64 |
| Bending length (cm) Flexural rigidity(mg-cm) | 25 × 200 mm | Paramount stiffness tester | BS 3356:1961 |
| Air permeability (m ³ /m ² /min.) | Template size | Shirley air permeability tester | BS 3321:1960 |
| Moisture regain (%) | 6" × 6" | Oven dry method and weighing balance | BS1051:1964 |

The change in physical properties of the fabric at various stages were studied.

Fabric count: Fabric count is the number of warp yarns (ends) and weft yarns (picks) per inch in a woven fabric. Paramount pick glass with pointer was used to determine the fabric count using ASTM-D123 test method. It was determined by counting the number of threads per square inch in the warp and weft directions at five places in the fabric. An average of five readings was taken as the fabric count.

Fabric weight per unit area: Samples free from selvedge were cut at random from controlled (scoured), treated and washed fabrics as per requirement of test method. The samples were conditioned and weighed separately on the Paramount Precision Scale for GSM (grams per square meter) using ASTM D3776-90 test method. An average of five readings was calculated.

Fabric thickness: The Shirley thickness tester was used to determine thickness of controlled, treated and washed samples with Guava leaves extract using BS 2544: 1967 test method. A fabric sample of 5"×5" was placed on the flat surface below pressure foot of the instrument without any fold. The pressure foot was lowered slowly upon the specimen until the pointer of the dial meter stopped moving further and the reading on the dial was recorded in mm. An average of five readings was calculated as the fabric thickness.

Fabric bulk: Fabric bulk (cm³/gm), which is a measure of specific volume (SV) of fabric, was also calculated for all treated fabrics along with control fabric using the formula:

$$\text{Bulk} = \frac{\text{Thickness in cm}}{\text{Weight g/cm}^2}$$

The size of fabric samples was 2"×2" and an average of five readings was taken.

Tensile Strength: Tensile strength is the ability of the fabric to withstand the load of force usually expressed as kilogram weight or pound weight. Tensile strength of three samples was determined on paramount universal tensile tester using ASTM-D1682-64 test method. The samples of size 12" × 2.5" were cut from warp and weft directions of the fabric. The yarns were unravelled from both sides to obtain sample of 2" width. The samples were mounted between the jaws at a distance of 10". The instrument was started by moving the lower jaw downwards till the sample broke. The breaking load was noted from the pointer on the dial meter. The tensile strength was calculated directly by observing the weight in kg in the dial meter. Average of five readings of the specimen from both directions (warp and weft) were taken.

Bending length and flexural rigidity: The bending length of three fabric samples was determined by the paramount stiffness tester using BS 3356: 1961 test method. Sample size of 25×200 mm (i.e. template size) were cut from fabrics in warp and weft directions with the aid of template. These were conditioned. Both template and sample were transferred to the platform with the fabric underneath, coinciding the zero mark of the scale and zero line engraved on the side of platform. The template was moved slowly over 41.5° slope along with the strip till the top of the specimen viewed in the mirror match in between both index lines. The bending length was read from the scale. Each sample was tested five times at each edge. An average of five readings was calculated for both the directions. All the samples were weighed, measured in length and width and their areas were calculated. Their weight per unit area was also determined in mg/sq.cm. Flexural rigidity was calculated using following formulae:

Flexural rigidity in warp direction (G1)

$$G1 = W \times C^2 \text{ mg-cm}$$

Flexural rigidity in weft direction (G2)

$$G2 = W \times C^2 \text{ mg-cm}$$

Where,

W = Weight per unit area of the fabric in milligrams per square centimeter.

C = The mean bending length for the respective direction.

Overall flexural rigidity (G)

$$G = G2 \times G1$$

Air permeability: The corresponding measurement of air permeability is that of the quantity of air passing through a surface of defined magnitude under a defined pressure difference and for a determined period of time. Prolific air permeability tester was used to find air permeability of the samples using BS3321:1960 test method at 10mm water column. The samples were taken and placed on the opening of the test cylinder under the clamping place. The clamps were tightened to the samples firmly. The motor of the vacuum pump was switched on and the valve was opened on the biggest rotameter till the required pressure

difference was obtained on the manometer. The flow rate on the scale fitted to rotameter tube against the top surface of the float was read. The experiment was repeated five times and mean was calculated. The experiment was conducted for, all the samples. Air permeability was calculated with the help of the following formula:

$$\text{Air permeability (AP)} = \frac{\text{Rate of air flow}}{\text{Exposed area}} \times 100$$

$$\text{AP} = k \times \text{Rotameter reading}$$

Where k is conversion factor i.e. = 0.01667 for 10mm water column

Moisture regain: Moisture regain is the weight of water in a material expressed as a percentage of the oven dry weight (the constant weight). Moisture regain was determined using BS: 1051:1964 test method. The oven was preheated for 10-15 minutes. The samples were weighed and placed in dry oven at $105 \pm 30^\circ\text{C}$ for 3 hours. The closed container was transferred to desiccator when the container and the specimen were cooled at room temperature. The samples were weighed and an average of five reading was calculated. The percentage of moisture regain was calculated using the following formula:

$$\text{Moisture regain (\%)} = \frac{\text{Original weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$

3.9 Determination of microbial population on treated samples

The microbial population (total colony forming units) of treated samples with inoculation of bacterium was determined quantitatively using AATCC-100 test method. The size of sample for determination of bacterium population was 2"×2".

3.10 Effectiveness of Guava leaves treatments with respect to washing

The CFUs of samples washed after all treatments and inoculated with cellulose degrading bacteria i.e. *Bacillus spp.* was determined quantitatively using AATCC-100 test method to find out the effectiveness of Guava leaves extract treatment with respect to washing (one wash cycle). **Subjecting the samples to one wash cycle was decided keeping the possibility of using Guava leaves extract as a renewable treatment at household level.** The fabric samples were then subjected to bacteria testing. Bacteria growth was analyzed by serial dilutions carried out after incubation. The effectiveness of the treatment was analyzed by washing all treated samples in the 'Launder-o-meter' by using standard ISO: 6330-1984E. At first the treated fabric were cut into samples of 100mm×25 mm and 4g/l soap solution was prepared with soap and 2g/l sodium carbonate (Na_2CO_3) (Saini, 2014).

Soap and sodium carbonate were mixed with water by taking 1:20 M:L:R. A homogeneous soap solution was obtained by stirring the solution for few minutes. The fabric samples and soap solutions were taken together in the steel cups of the 'Launder-o-meter'.

The cups were fastened inside the instrument and the lid was closed. The time was set 20 minutes and temperature 30°C. After the completion of washing the samples were air dried.

The washed fabric samples were inoculated to find out the effectiveness the Guava leaves extract with respect to washing.

3.11 Statistical Analysis

Data for Peach leaves treated fabric samples were coded, tabulated and analyzed by the application of statistical tools as given below.

Percentage: Changes in all physical properties after treatment and washing were calculated in percentages.

Mean: Mean values of CFU counts with dilution factors (10^7 and 10^8) and physical properties of treated and washed samples were calculated for determination of microbial population and assessment of efficacy of treatments respectively. Formula used was:

$$\text{Arithmetic mean} = \sum \frac{X}{n}$$

Where,

X = sum of all the observations

n = total number of observations

Standard Deviation: Standard deviation was used to calculate the error in the mean of different treatments and in different concentrations.

$$= \sqrt{\sum X_i^2 / n}$$

Standard Error: Standard error was used to calculate the error in the mean of physical properties of samples. The following formula was used:

$$SE_1 = \frac{s}{\sqrt{n}}$$

T-test: It was applied to test the significance of change in the physical properties, after washing and after cellulose degrading bacteria inoculation in samples between the different treatments.

The following formula was used:

$$t = \frac{\bar{d}}{S/\sqrt{n}} \text{ with } (n - 1) \text{ degrees of freedom}$$

Where:

\bar{d} = The mean of the difference of untreated control and treated fabric

n = Number of observations

S = Standard deviation of the difference computed by usual formula

The present study was undertaken to impart Guava leaves extract treatment on cotton fabric for antibacterial activity against growth of cellulose degrading bacteria i.e. *Bacillus spp.* Extraction of Guava leaves was done for two different time periods separately, the time period having greater yield percentage was selected for extraction. Three different concentrations of Guava leaves extract was screened for its antibacterial properties and one concentration exhibiting strong zone of antibacterial property was selected for application of treatment with exhaust method. The efficacy of treatment with regard to antibacterial activity was studied by using standard methods. The effect of treatment on the physical properties of fabric as well as wash durability of treated fabrics was also studied. The results obtained are being presented under the following sub heads:

- 4.1 Preliminary data of the selected cotton fabric
- 4.2 Effect of preparatory processes on physical properties of grey fabric
- 4.3 Analysis and selection of Guava leaves extract concentration
- 4.4 Determination of bacterial resistance of Guava leaves extract treatment and its effect on physical properties
- 4.5 Efficacy of Guava leaves extract treatment with respect to washing and its effect on physical properties
- 4.6 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

4.1 Preliminary data of the selected cotton fabric

Cotton woven fabric exhibiting light to medium fabric weight and suitable for apparel during summer season was collected in grey state. Fabric count, weight and thickness of selected fabric was evaluated and presented in Table 2. The data in Table 2 revealed that fabric count of grey cotton woven fabric was 31 x 22 ends and picks per sq. inch. The fabric weighed 141g/m² with 0.254 mm thickness.

Table 2: Preliminary data of selected cotton woven fabric

| Fabric properties | Fabric count (ends and picks/sq. inch) | Fabric weight (g/m ²) | Fabric thickness (mm) |
|-------------------|---|--------------------------------------|--------------------------|
| Cotton fabric | 31 × 22 | 141 | 0.254 |

4.2 Effect of preparatory processes on physical properties of grey fabric

Desizing and scouring involves removal of starch and impurities present in the fabric to make it absorbent for textile processing and simultaneously may alter the physical properties of the fabric. Grey cotton woven fabric was subjected to desizing followed by scouring. The pre-treated cotton woven fabric was studied for changes in physical properties due to desizing and scouring.

It was clearly observed from the data in Table 3 that the weight and fabric count in both warp and weft directions of grey fabric was $141 \pm 0.89 \text{ g/m}^2$, 31 ± 0.45 ends and picks per sq. inch and 22 ± 0.02 ends and picks per sq. inch which after desizing and scouring increased to $149 \pm 0.44 \text{ g/m}^2$, 36 ± 0.05 ends and picks per sq. inch and 25 ± 0.30 ends and picks per sq. inch with 5.67, 16.12 and 13.63 percent increase and 7.01, 5.68 and 4.23 t-values, respectively. The gain in weight and fabric count in both warp and weft directions were found to be significant at 1% level of significance. The tensile strength in both warp and weft directions of grey fabric was $21.7 \pm 0.08 \text{ kg}$ and $25.96 \pm 0.09 \text{ kg}$ which reduced to $19.43 \pm 0.21 \text{ kg}$ and $23.98 \pm 0.38 \text{ kg}$ after desizing and scouring thus showing percent reduction of 10.46 and 7.62 with 9.38 and 4.79 t-values, respectively. The decrease in tensile strength of the fabric samples both in warp and weft directions was significant at 1% level of significance.

The thickness and bulk of grey fabric was observed to be $0.254 \pm 0.04 \text{ mm}$ and $2.80 \pm 0.02 \text{ cm}^3/\text{gm}$ which increased to $0.288 \pm 0.003 \text{ mm}$ and $2.93 \pm 0.03 \text{ cm}^3/\text{gm}$ after desizing and scouring with percent increase of 13.38 and 4.64 with 4.54 and 3.51 t-values, respectively. The increase in thickness and bulk after desizing and scouring was significant at 5% level of significance. The air permeability of grey fabric was $197.43 \pm 0.40 \text{ m}^3/\text{m}^2/\text{min}$. This decreased to $194.3 \pm 0.59 \text{ m}^3/\text{m}^2/\text{min}$. There was 1.58 percent reduction with 3.14 t-value. The decrease in air permeability of the desized and scoured sample was significant at 5 % level of significance.

Table 3: Effect of preparatory processes on physical properties of grey fabric

| Physical Properties | | Fabric | | Percent change | t-value |
|--|------|---------------------|------------------------------------|----------------|---------|
| | | Grey Mean \pm S.E | Desized and Scoured Mean \pm S.E | | |
| Weight per unit area (g/m^2) | | 141 ± 0.89 | 149 ± 0.44 | +5.67 | 7.01** |
| Fabric count (ends and picks per sq. inch) | Warp | 31 ± 0.45 | 36 ± 0.05 | +16.12 | 5.68** |
| | Weft | 22 ± 0.02 | 25 ± 0.30 | +13.63 | 4.23** |
| Tensile strength (kg) | Warp | 21.7 ± 0.08 | 19.43 ± 0.21 | -10.46 | 9.38** |
| | Weft | 25.96 ± 0.09 | 23.98 ± 0.38 | -7.62 | 4.79** |
| Thickness (mm) | | 0.254 ± 0.04 | 0.288 ± 0.003 | +13.38 | 4.54* |
| Bulk (cm^3/gm) | | 2.80 ± 0.02 | 2.93 ± 0.03 | +4.64 | 3.51* |
| Air permeability($\text{m}^3/\text{m}^2/\text{min}$) | | 197.43 ± 0.40 | 194.3 ± 0.59 | -1.58 | 3.14* |
| Bending length(cm) | Warp | 4.30 ± 0.17 | 4.08 ± 0.17 | -5.11 | 1.38 |
| | Weft | 3.48 ± 0.14 | 3.44 ± 0.07 | -1.14 | 0.20 |
| Flexural rigidity (mg-cm) | | 5.58 ± 0.11 | 5.43 ± 0.04 | -2.68 | 1.24 |
| Moisture regain (%) | | 0.21 ± 0.09 | 0.18 ± 0.09 | -14.28 | 1.67 |

**Significant at 1%level of significance

*Significant at 5%level of significance

Decrease was also observed in bending length both in warp from 4.30 ± 0.17 to $4.08 \pm 0.17 \text{ cm}$ and weft from 3.48 ± 0.14 to $3.44 \pm 0.07 \text{ cm}$ directions, flexural rigidity from 5.58 ± 0.11 to $5.43 \pm 0.04 \text{ mg-cm}$ and moisture regain from 0.21 ± 0.09 to $0.18 \pm 0.09 \%$ after

desizing and scouring. The percent reduction in bending length in both warp and weft directions, flexural rigidity and moisture regain were 5.11, 1.14, 2.68 and 14.28 with 1.38, 0.20, 1.24 and 1.67 t-values. These were found to be non-significant.

It was discernible from the results presented in Table 3 that after desizing and scouring there was significant increase in weight per unit area, fabric count in warp and weft directions, thickness and bulk while there was significant decrease in tensile strength in both the directions and air permeability. The changes in bending length in both warp and weft directions, flexural rigidity and moisture regain were found to be non-significant.

4.3 Analysis and selection of Guava leaves extract concentration

The results of the analysis of cold aqueous extract of Guava leaves in respect of yield percentage and antibacterial property for selection of time period for extraction and its concentration under study are given below:

4.3.1 Determination of yield percentage of Guava leaves extract

Data presented in Table 4 and Fig 1 showed the yield obtained by cold maceration extraction process of Guava leaves at different time periods. Results revealed that the yield obtained for 12 hours was 6 g and for 24 hours was 15 g. Thus, the yield percentage of Guava leaves was found to be higher for 24 hours (15%) as compared to yield percentage for 12 hours (6%). The yield percentage increased with an increase in the time period of maceration i.e. 6 percent for 12 hours and 15 percent for 24 hours. Therefore, 24 hour time period for extraction was selected and continued for further research work.

Table 4: Determination of yield percentage of Guava leaves extract at different time periods

| Plant source | Method of extraction | Time period (hours) | Weight of the air dry powder (g) | Weight of the extract (g) | Yield percentage (%) |
|--------------|----------------------|---------------------|----------------------------------|---------------------------|----------------------|
| Guava leaves | Maceration | 12 | 100 | 6 | 6 |
| | | 24 | 100 | 15 | 15 |

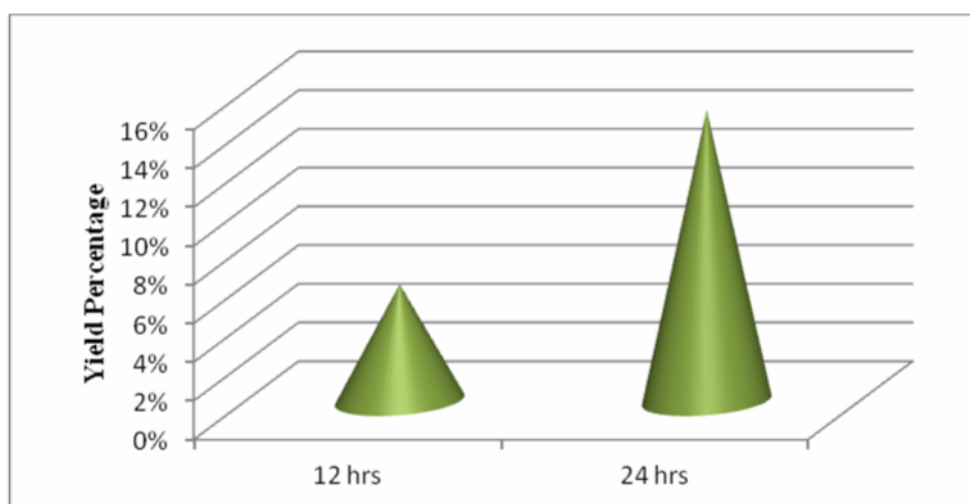


Fig. 1: Determination of yield percentage of Guava leaves extract at different time periods

4.3.2 Antibacterial property of Guava leaves extract at different concentrations

Antibacterial property of Guava leaves extract at different concentrations was tested by using agar well diffusion method against cellulose degrading bacteria i.e. *Bacillus spp.* The concentrations were 1mg/ml, 3mg/ml and 5mg/ml. The results were obtained in terms of zone of inhibition in mm and interpreted as no inhibition, weak, moderate and strong.

The data in Table 5 and Fig 2 highlighted that sterilized distilled aqueous kept as control had no inhibition. The concentrations 1 mg/ml and 3mg/ml exhibited moderate zones of inhibition i.e. 10mm and 12 mm, respectively while 5 mg/ml concentration had a strong zone of inhibition i.e. 19 mm against *Bacillus spp.*

Thus, with an increase in the concentration of Guava leaves extract, its zone of inhibition indicating antibacterial property was also increased. Therefore, 5 mg/ml concentration had a strong zone of inhibition i.e. 19 mm against *Bacillus spp.* was selected and continued for further research work.

Table 5: Antibacterial property of Guava leaves extract at different concentrations

| Guava leaves extract Concentrations | 1 mg/ml | 3 mg/ml | 5 mg/ml |
|--|---------------|---------|---------|
| Zone of inhibition (mm) * | 10 | 12 | 19 |
| Control (sterilized distilled aqueous) | No Inhibition | | |

*No activity (-mm), weak (<6mm), moderate (7-12mm) and strong activity (>12mm).

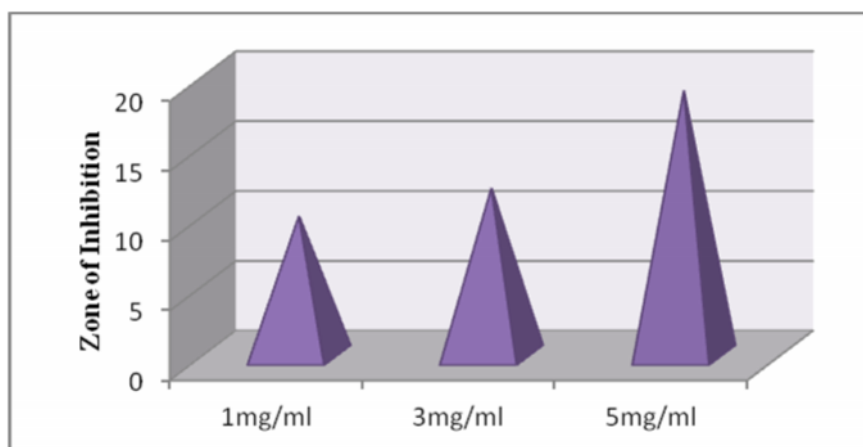


Fig. 2: Antibacterial property of Guava leaves extract at different concentrations

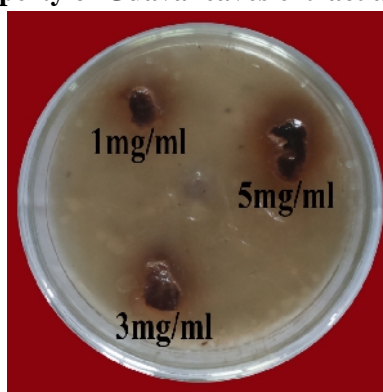
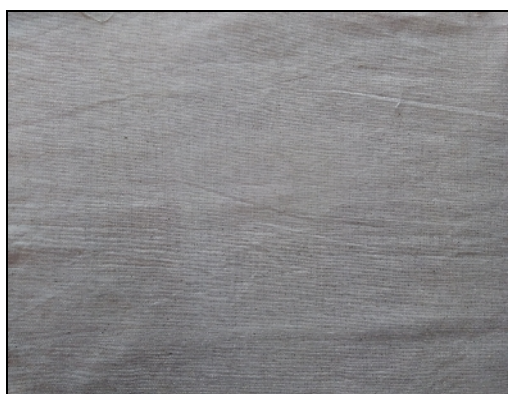


Plate 10: Zone of inhibition of Guava leaves extract at different concentrations



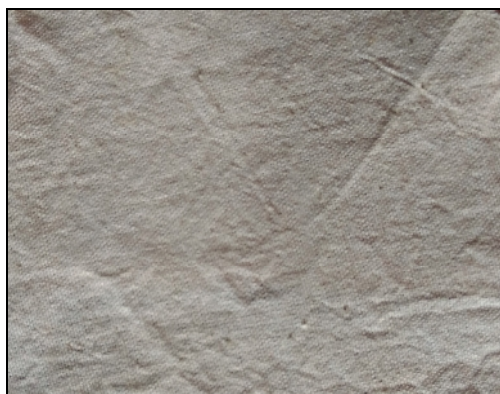
GREY COTTON WOVEN FABRIC



DESIZED AND SCOURED FABRIC



TREATED FABRIC



WASHED FABRIC

Plate 11 : Grey, controlled, treated and washed fabrics

4.4 Determination of bacterial resistance of Guava leaves extract treatment and its effect on physical properties of fabric

The results related to the efficacy of Guava leaves extract treatment against antibacterial activity and its effect on physical properties of fabric is given below:

4.4.1 Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.*

The efficacy of Guava leaves extract treatment against growth of cellulose degrading bacteria i.e. *Bacillus spp.* on treated samples, was counted quantitatively by AATCC-100 test method. The antibacterial activity of treated cotton woven fabric inoculated with *Bacillus spp.* was compared to its controlled sample by calculating the percent reduction in bacterial count. The observations were recorded soon after 24 hours of inoculation of *Bacillus spp.* on the same day and also on 7th, 14th, 21st and 28th days of inoculation with dilution factors 10⁷ and 10⁸. Mean values of 10⁷ and 10⁸ dilution factors were calculated.

The data presented in Table 6 and Fig 3 indicated the percent reduction and bacterial colony counts of *Bacillus spp.* on subsequent days of inoculation. The percent reduction was compared with controlled sample i.e. untreated desized and scoured sample. Confluent lawn of growth was observed on controlled sample.

There was 100 percent reduction on 0 day soon after 24 hours of inoculation i.e. no bacterial growth was observed in treated fabric with mean CFUs in 10^7 and 10^8 dilution factors. The percent reduction on 7th day was 93 percent with 7×10^8 mean CFUs in 10^7 and 10^8 dilution factors. The percent reduction on 14th, 21st and 28th day of inoculation was found to be 86, 77 and 67 with 1.4×10^9 , 2.3×10^9 and 3.3×10^9 mean CFUs in 10^7 and 10^8 dilution factors respectively.

The antibacterial treatment with 5g/l concentration of Guava leaves extract with dilution factor (10^7 and 10^8) was cent percent effective after 24 hours of inoculation of *Bacillus spp.* After 24 hours percent bacterial reduction gradually decreased and on 28th day it reduced to 67%. Thus, there was subsequent percent reduction in bacterial count with increase in incubation period.

Table 6: Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.*

| Cellulose degrading bacteria | <i>Bacillus spp.*</i> | | | | |
|------------------------------|-----------------------------------|---------------------|----------------------|----------------------|----------------------|
| Incubation Period | 0 Day (soon after 24 hours) | 7 th Day | 14 th Day | 21 th Day | 28 th Day |
| Treated fabric | 0 | 7×10^8 | 1.4×10^9 | 2.3×10^9 | 3.3×10^9 |
| Percent reduction (%) | 100 | 93 | 86 | 77 | 67 |
| Controlled fabric | Confluent growth | | | | |

*Dilutions Mean of 10^7 & 10^8 CFU/ml

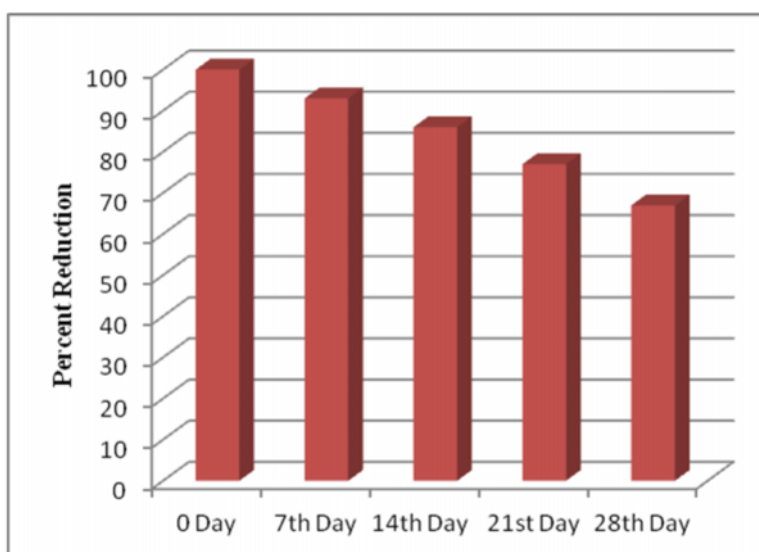


Fig. 3: Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.* at different incubation periods



Plate 12: Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.* at different incubation periods

4.4.2 Effect of Guava leaves extract treatment on physical properties of fabric

It was observed from the data in Table 7 that the weight per unit area of the fabric after Guava leaves extract treatment increased from 149 ± 0.44 to 154.6 ± 0.51 g/m² with 3.75 percent increase and 6.89 t-value. The gain in weight per unit area was significant at 1% level of significance. The bulk of controlled fabric sample (desized and scoured) was 2.93 ± 0.03 cm³/gm which increased to 3.07 ± 0.03 cm³/gm after treatment with 4.77 percent and 6.32 t-value. The increase in bulk after treatment was found significant at 1% level of significance.

The air permeability of controlled fabric was found to be 194.3 ± 0.59 m³/m²/min which decreased to 191.35 ± 0.45 m³/m²/min after treatment, similarly the tensile strength of controlled fabric sample in warp direction was observed to be 19.43 ± 0.21 kg which decreased to 17.68 ± 0.37 kg after treatment. The air permeability and tensile strength in warp direction showed a reduction of 1.51 percent and 9.00 percent with 5.50 and 5.77 t-values. The decrease in air permeability and tensile strength in warp direction was significant at 1% level of significance, respectively. The tensile strength of controlled fabric in weft direction was found to be 23.98 ± 0.38 kg which decreased to 21.98 ± 0.5 kg after treatment, similarly the flexural rigidity of controlled fabric was observed to be 5.43 ± 0.04 mg-cm which decreased to 5.07 ± 0.10 mg-cm after treatment. The changes in weft direction of tensile strength and flexural rigidity showed a reduction of 8.34 percent and 6.62 percent with 3.20 and 3.55 t-value. The changes in tensile strength in weft direction and flexural rigidity were significant at 5% level of significance, respectively.

There was percent change in fabric count in both warp from 36 ± 0.05 to 38 ± 0.06 ends and picks per sq. inch and weft from 25 ± 0.30 to 26 ± 0.30 ends and picks per sq. inch directions, thickness from 0.288 ± 0.003 to 0.306 ± 0.008 mm, bending length in both warp from 4.08 ± 0.17 to 3.74 ± 0.09 cm and weft from 3.44 ± 0.07 to 3.18 ± 0.08 cm direction and moisture regain from 0.18 ± 0.09 to 0.16 ± 0.01 % of the samples after treatment with Guava

leaves extract with +5.55, +4.00, +6.25, -8.33, -7.55 and -11.11 percent change and 1.98, 1.14, 2.09, 1.33, 1.76 and 2.52 t-values, respectively. The percent changes in fabric count in both warp and weft directions, thickness, bending length in both warp and weft direction and moisture regain were found to be non- significant.

Thus, it is unmistakably deferred that weight per unit area and bulk increased significantly while tensile strength in both warp and weft directions, flexural rigidity and air permeability decreased significantly after the take-up of the treatment. The changes in fabric count in both warp and weft directions, thickness, bending length in both warp and weft direction and moisture regain were found to be non- significant.

Table 7: Effect of Guava leaves extract treatment on physical properties of fabric

| Physical properties | Fabric | | Percent change | t-value | |
|--|---------------------------|------------------------|-------------------|---------|--------|
| | Controlled Mean \pm S.E | Treated Mean \pm S.E | | | |
| Weight per unit area (g/m ²) | 149 \pm 0.44 | 154.6 \pm 0.51 | +3.75 | 6.89** | |
| Bulk (cm ³ /gm) | 2.93 \pm 0.03 | 3.07 \pm 0.03 | +4.77 | 6.32** | |
| Air permeability (m ³ /m ² /min) | 194.3 \pm 0.59 | 191.35 \pm 0.45 | -1.51 | 5.50** | |
| Tensile strength (kg) | Warp | 19.43 \pm 0.21 | 17.68 \pm 0.37 | -9.00 | 5.77** |
| | Weft | 23.98 \pm 0.38 | 21.98 \pm 0.5 | -8.34 | 3.20* |
| Flexural rigidity (mg-cm) | | 5.43 \pm 0.04 | 5.07 \pm 0.10 | -6.62 | 3.55* |
| Fabric count (ends and picks per sq. inch) | Warp | 36 \pm 0.05 | 38 \pm 0.06 | +5.55 | 1.98 |
| | Weft | 25 \pm 0.30 | 26 \pm 0.30 | +4.00 | 1.14 |
| Thickness (mm) | | 0.288 \pm 0.003 | 0.306 \pm 0.008 | +6.25 | 2.09 |
| Bending length(cm) | Warp | 4.08 \pm 0.17 | 3.74 \pm 0.09 | -8.33 | 1.33 |
| | Weft | 3.44 \pm 0.07 | 3.18 \pm 0.08 | -7.55 | 1.76 |
| Moisture regain (%) | | 0.18 \pm 0.09 | 0.16 \pm 0.01 | -11.11 | 2.52 |

**Significant at 1% level of significance

* Significant at 5% level of significance

4.5 Efficacy of Guava leaves extract treatment with respect to washing and its effect on physical properties

The wash durability of Guava leaves extract treatment with respect to antibacterial activity was assessed after washing (one wash cycle) by using ISO: 6330-1984E. The effect of washing on physical properties of fabric were also analysed after one wash by using test standard methods. The results have been presented in Table 8 and 9.

4.5.1 Efficacy of Guava leaves extract treatment with respect to washing

The effectiveness of Guava leaves extract treatment after one wash was analysed against antibacterial activity of cellulose degrading bacteria i.e. *Bacillus spp.* The percent reduction was checked on 0 day (soon after 24 hours) of inoculation, 7th day, 14th day, 21st day and 28th day of inoculation with dilution factors 10⁷ and 10⁸. Mean values of 10⁻⁷ and 10⁻⁸ dilution factors were calculated. The unwashed samples treated with Guava leaves extract were the controlled samples for comparing the efficacy of treatment after washing and inoculated with *Bacillus spp.* The growth of CFUs in washed samples was compared to the unwashed sample.

Table 8: Efficacy of Guava leaves extract treatment with respect to washing

| Cellulose Degrading Bacteria | <i>Bacillus spp.*</i> | | | | | | | | | |
|------------------------------|--------------------------------|--------|---------------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|
| Incubation Period | 0 Day (soon after 24 hours) | | 7 th Day | | 14 th Day | | 21 th Day | | 28 th Day | |
| Fabric | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed |
| Colony count | 0 | 0 | 7×10^8 | 1.0×10^9 | 1.4×10^9 | 1.8×10^9 | 2.3×10^9 | 2.7×10^9 | 3.3×10^9 | 4.3×10^9 |
| Percent reduction (%) | 100 | 100 | 93 | 90 | 86 | 82 | 77 | 73 | 67 | 64 |
| Controlled | Confluent growth | | | | | | | | | |

*Dilutions Mean of 10^7 & 10^8 CFU/ml

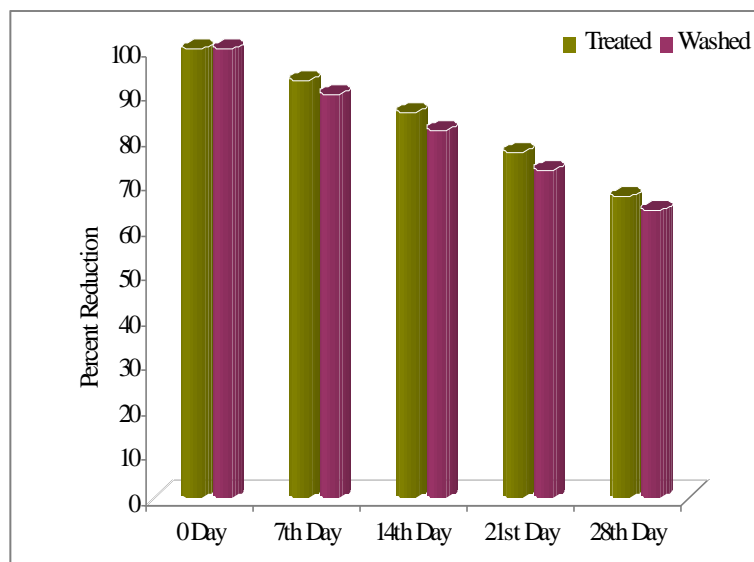


Fig. 4: Efficacy of Guava leaves extract treatment with respect to washing at different incubation periods



Plate 13: Efficacy of Guava leaves extract treatment with respect to washing at different incubation periods

The data regarding efficacy of Guava leaves extract treatment against washing in Table 8 depicted that on 0 day (soon after 24 hours of inoculation) there was no bacterial growth.

On 7th day the percent reduction was observed to be 90 percent with 1.0×10^9 mean CFUs in 10^7 and 10^8 dilution factors in washed fabric against 93 percent reduction with 7×10^8 mean CFUs in 10^7 and 10^8 dilution factors in treated unwashed fabric. On 14th day of inoculation the percent reduction was recorded to be 82 percent with 1.8×10^9 mean CFUs in 10^7 and 10^8 dilution factors in washed fabric against 86 percent reduction with 1.4×10^9 mean CFUs in 10^7 and 10^8 dilution factors in treated unwashed fabric.

On 21st day of inoculation the percent reduction was recorded to be 73 percent with 2.7×10^9 mean CFUs in 10^7 and 10^8 dilution factors in washed fabric against 77 percent reduction with 2.3×10^9 mean CFUs in 10^7 and 10^8 dilution factors in treated unwashed fabric. On 28th day of inoculation the percent reduction was recorded to be 64 percent with 4.3×10^9 mean CFUs in 10^7 and 10^8 dilution factors in washed fabric against 67 percent reduction with 3.3×10^9 mean CFUs in 10^7 and 10^8 dilution factors in treated unwashed fabric.

The Guava leaves extract treated samples exhibited cent percent wash durability after 24 hours of inoculation in washed samples (one wash). After 24 hours it gradually decreased and on 28th day it reduced to 64%. Thus, there was continuous decrease in the antibacterial activity with an increase in incubation period after washing but even then the antibacterial treatment was actively retained in washed samples even on 28th day.

4.5.2 Effect of washing on physical properties of fabric

The effect of Guava leaves extract treatment on physical properties was analysed by using standard test methods. The effect of washing on physical properties of fabric was analysed after one wash by using standard test methods.

Table 9: Effect of washing on physical properties of fabric

| Physical Properties | Fabric | | Percent change | t-value | |
|---|------------------------|-----------------------|------------------|---------|-------|
| | Treated Mean \pm S.E | Washed Mean \pm S.E | | | |
| Weight per unit area (g/m^2) | 154.6 ± 0.51 | 148.2 ± 0.37 | -4.13 | 7.87** | |
| Bulk (cm^3/gm) | 3.07 ± 0.03 | 2.78 ± 0.01 | -9.44 | 9.72** | |
| Air permeability ($\text{m}^3/\text{m}^2/\text{min}$) | 191.35 ± 0.45 | 195.12 ± 0.59 | +1.97 | 4.36* | |
| Tensile strength (kg) | Warp | 17.68 ± 0.37 | 16.32 ± 0.19 | -7.69 | 2.78* |
| | Weft | 21.98 ± 0.5 | 19.82 ± 0.08 | -9.82 | 3.71* |
| Flexural rigidity (mg-cm) | 5.07 ± 0.10 | 4.63 ± 0.06 | -8.6 | 3.06* | |
| Thickness (mm) | 0.306 ± 0.008 | 0.288 ± 0.003 | -5.88 | 2.09 | |
| Bending length(cm) | Warp | 3.74 ± 0.09 | 3.44 ± 0.09 | -8.02 | 1.82 |
| | Weft | 3.18 ± 0.08 | 2.96 ± 0.04 | -6.91 | 2.00 |
| Fabric count | Warp | 38 ± 0.06 | 36 ± 0.09 | -5.26 | 1.90 |
| | Weft | 26 ± 0.30 | 25 ± 0.04 | -3.84 | 1.36 |
| Moisture regain (%) | 0.16 ± 0.01 | 0.17 ± 0.003 | +6.25 | 1.00 | |

**Significant at 1% level of significance

*Significant at 5% level of significance

The data in Table 9 revealed that the weight per unit area and bulk of treated fabric was found to be $154.6 \pm 0.51 \text{ g/m}^2$ and $3.07 \pm 0.03 \text{ cm}^3/\text{gm}$ which reduced to $148.2 \pm 0.37 \text{ g/m}^2$ and $2.78 \pm 0.01 \text{ cm}^3/\text{gm}$ after washing respectively. The weight per unit area and bulk of the fabric showed a reduction of 4.13 percent and 9.44 percent with 7.87 and 9.72 t-values. The change in weight per unit area and bulk was significant at 1% level of significance.

The air permeability of treated fabric was $191.35 \pm 0.45 \text{ m}^3/\text{m}^2/\text{min}$ which increased to $195.12 \pm 0.59 \text{ m}^3/\text{m}^2/\text{min}$ after washing. There was 1.97 percent increase with 4.36 t-value in air permeability. The change in air permeability was significant at 5% level of significance. The tensile strength in both warp and weft directions and flexural rigidity of treated fabric were $17.68 \pm 0.37 \text{ kg}$, $21.98 \pm 0.5 \text{ kg}$ and $5.07 \pm 0.10 \text{ mg-cm}$ which reduced to $16.32 \pm 0.19 \text{ kg}$, $19.82 \pm 0.08 \text{ kg}$ and $4.63 \pm 0.06 \text{ mg-cm}$ with a percent reduction of 9.82 percent, 7.69 percent and 8.6 percent with 2.78, 3.71 and 3.06 t-values respectively. The changes in tensile strength in both warp and weft directions and flexural rigidity were significant at 5% level of significance.

There was reduction in thickness from 0.306 ± 0.008 to $0.288 \pm 0.003 \text{ mm}$, bending length in both warp from 3.74 ± 0.09 to $3.44 \pm 0.09 \text{ cm}$ and weft from 3.18 ± 0.08 to $2.96 \pm 0.04 \text{ cm}$ directions, fabric count in both warp from 38 ± 0.06 to 36 ± 0.09 ends and picks per sq. inch and weft from 26 ± 0.30 to 25 ± 0.04 ends and picks per sq. inch directions with 5.88, 8.02, 6.91, 5.26 and 3.84 percent change and 2.09, 1.82, 2.00, 1.90 and 1.36 t-values and gain in moisture regain from 0.16 ± 0.01 to $0.17 \pm 0.003 \%$ with 6.25 percent change and 1.00 t-value, respectively in treated samples after washing. The changes in thickness, bending length, fabric count in both warp and weft direction and moisture regain were found to be non-significant.

Therefore, it is clearly evident that after washing significant decrease was observed in weight per unit area, bulk, tensile strength in both warp and weft directions and flexural rigidity while there was significant increase in air permeability. The changes in thickness, bending length in both warp and weft directions, fabric count in both warp and weft direction and moisture regain were found to be non-significant.

4.6 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

An attempt has been made to compare the physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing to analyse the effect of these treatments. The analysis is done on the basis of data in terms of percent change and t-value indicated in the Table 3, 7 and 9 and mean values depicted from the Fig 5-13.

The data regarding percent change and t-value presented in Table 3, 7 and 9 & mean values depicted in Fig 5, 6 7 and 8 in respect of weight per unit area, thickness, bulk and fabric count in both warp and weft direction revealed that there was remarkable increase in

weight per unit area (+5.67, +3.75), thickness (+13.38, +6.25), bulk (+4.64, +4.77) and fabric count both in warp(+16.12, +5.55) and weft (+13.63, +4.00) properties after the application of the Guava leaves extract treatment while these properties i.e. weight per unit area (-4.13), thickness (-5.88), bulk (-9.44) and fabric count both in warp (-5.26) and weft (-3.84) were decreased after washing.

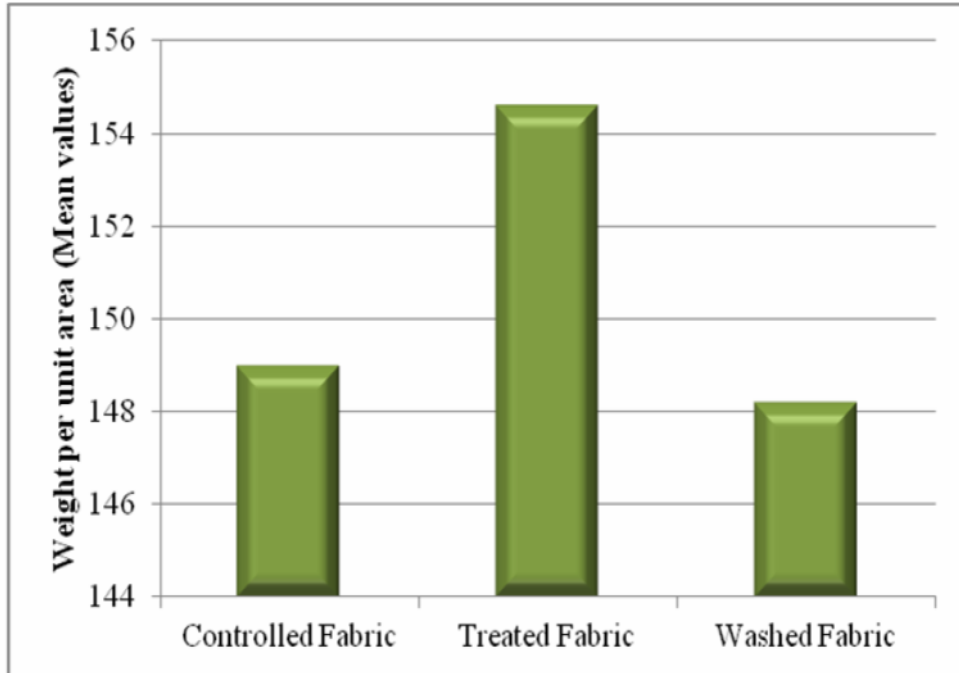


Fig. 5: Comparative analysis of weight per unit area of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

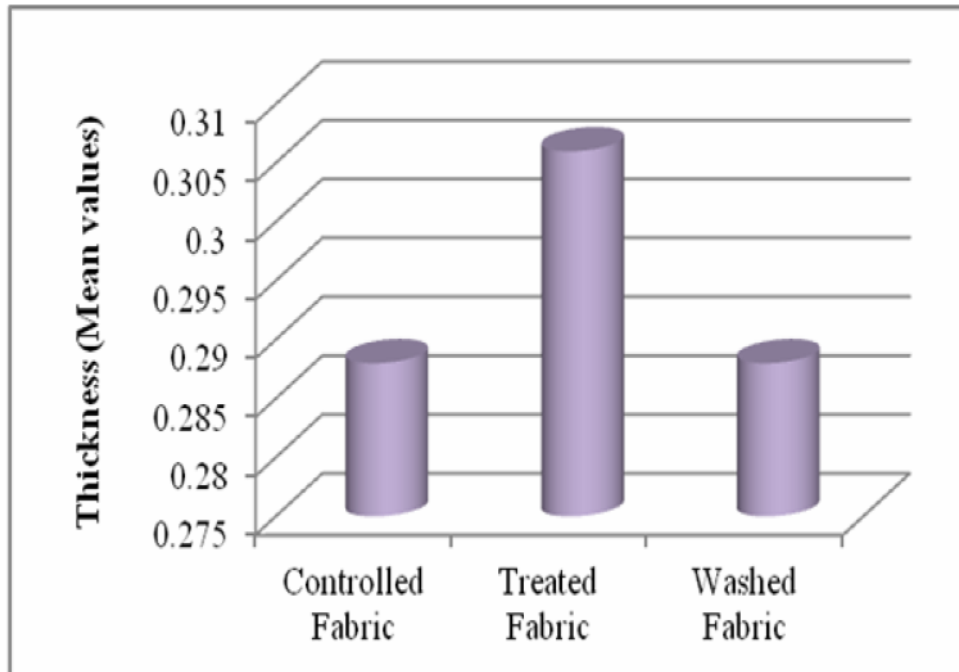


Fig 6: Comparative analysis of thickness of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

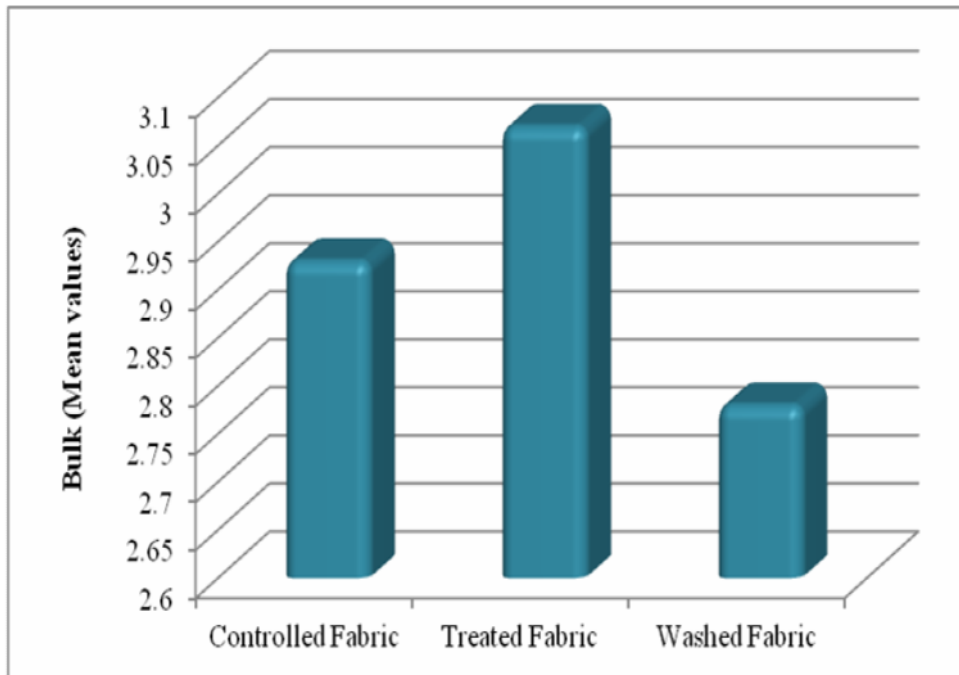


Fig. 7: Comparative analysis of bulk of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

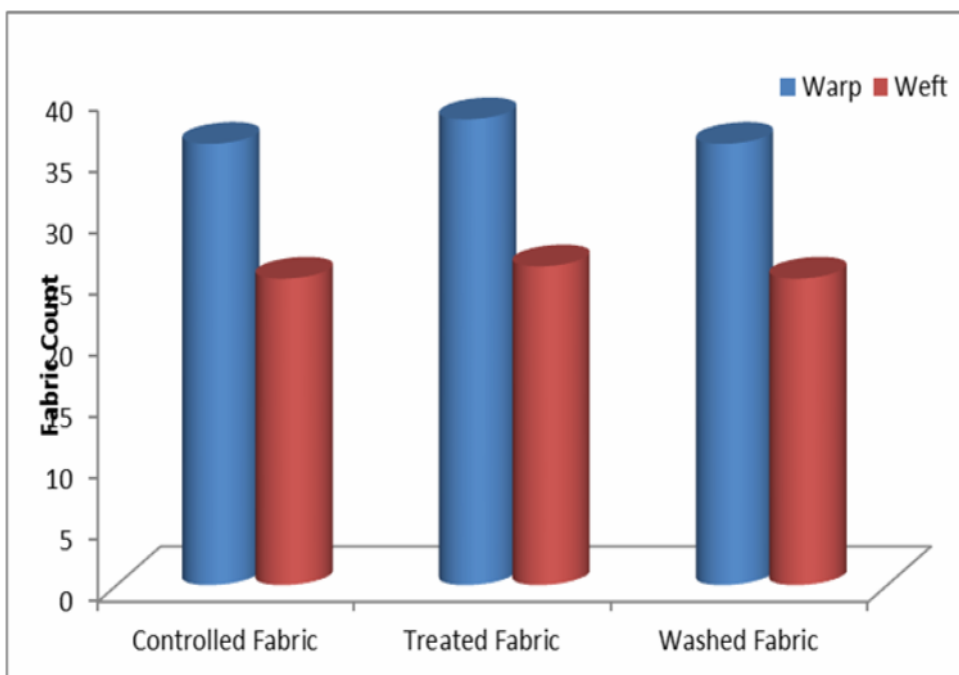


Fig. 8: Comparative analysis of fabric count of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

The data presented in Table 3, 7 and 9 and Fig 9,10 and 11 indicated that the mean values of flexural rigidity (-2.68, -6.62, -8.6), tensile strength in both warp (-10.46, -9.00,- 7.69) and weft (-7.62, -8.34, -9.82) directions and bending length in both warp(-5.11, -8.33,- 8.02) and weft(-1.14, -7.55, -6.91) directions reduced after the Guava leaves extract treatment and continuously decreased after washing also.

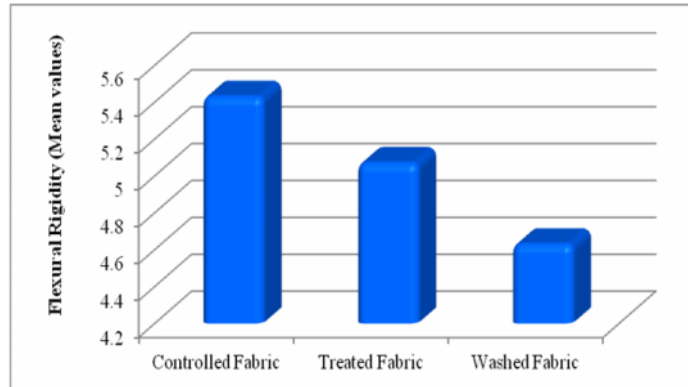


Fig. 9: Comparative analysis of flexural rigidity of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

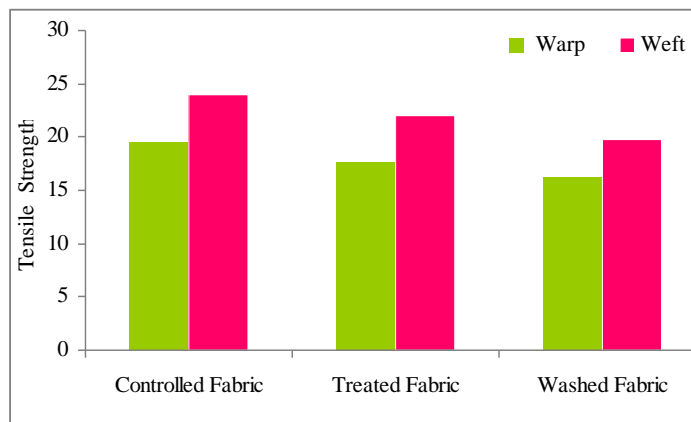


Fig. 10: Comparative analysis of tensile strength in warp and weft direction of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

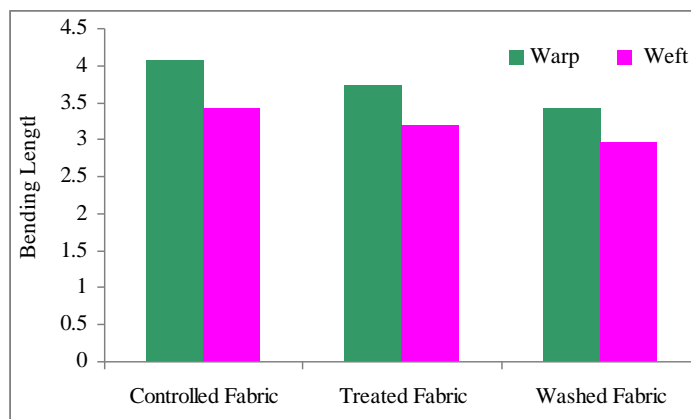


Fig. 11: Comparative analysis of bending length in warp and weft of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

It is evident from the data in Table 3, 7 and 9 and Fig 12 and 13 that there was decrease in air permeability (-1.58 and -1.51) and moisture regain (-14.28 and -11.11) of cotton woven fabric after treatment and but after washing air permeability (+1.97) and moisture regain (+6.25) increased.

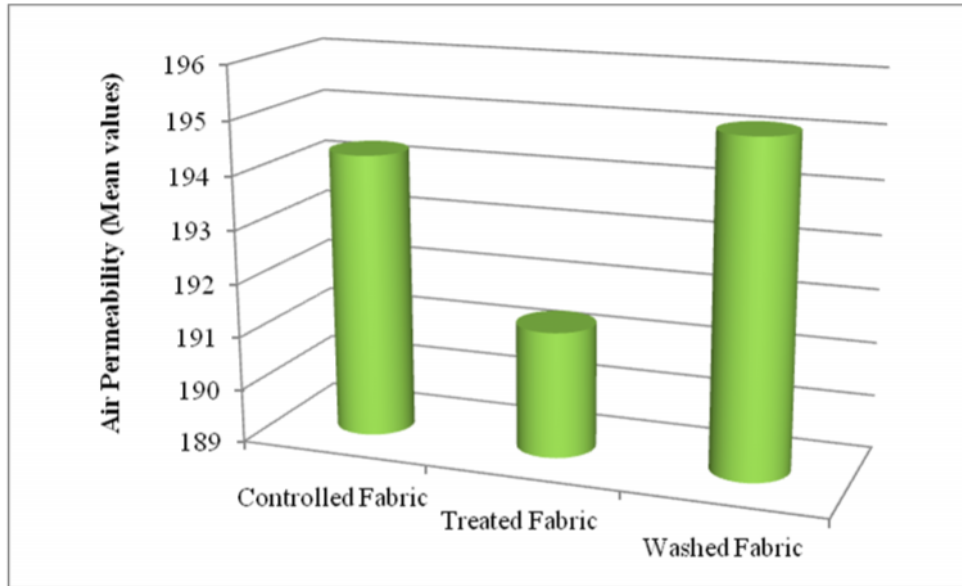


Fig. 12: Comparative analysis of air permeability of cotton fabric after preparatory process, Guava leaves extract treatment and washing

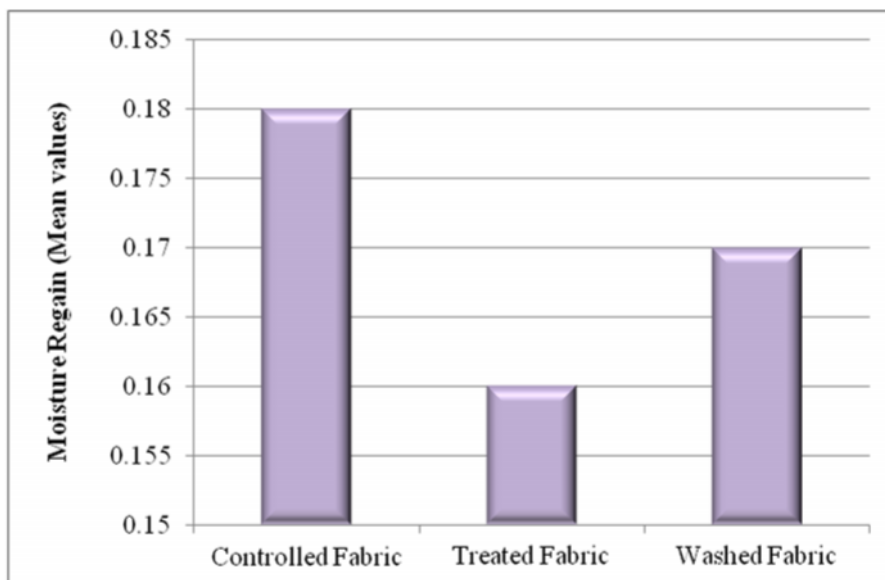


Fig. 13: Comparative analysis of moisture regain of cotton fabric after preparatory process, Guava leaves extract treatment and washing

With increasing health problems related to microbes, consumers are becoming very conscious for textiles that are hygienic. Hygienic textiles and congenial micro environment are prime requirements for human beings to live comfortably. Cotton though comfortable and hygienic fabric due to its good absorbency, but vulnerable to microbial attack. Among different natural materials, plants show the highest potential to be used as antimicrobial agents and are applied on textile materials to develop the microbial resistant textiles with multiple applications. Moreover eco-friendly textiles are in demand due to stringent safety laws. Therefore, Guava leaves extract was selected to impart herbal antimicrobial treatment on cotton fabric. To achieve the objectives desized and scoured cotton fabric was treated with Guava leaves extract. The efficacy of the treatment against antibacterial activity and wash durability was assessed. Changes in physical properties of grey, treated samples and washed samples were also examined. Discussion regarding findings of the study is presented under following heads:

- 5.1 Analysis and selection of Guava leaves extract concentration
- 5.2 Effect of preparatory processes on physical properties of grey fabric
- 5.3 Determination of bacterial resistance of Guava leaves extract treatment and its effect on physical properties
- 5.4 Efficacy of Guava leaves extract treatment with respect to washing and its effect on physical properties
- 5.5 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

5.1 Analysis and selection of Guava leaves extract concentration

Analysis of Guava leaves extract for the application on cotton fabric was done under different parameters i.e. yield percentage obtained and antibacterial property at different concentrations.

5.1.1 Determination of yield percentage of Guava leaves extract

To determine the yield percentage of Guava leaves extract at two different time periods, cold aqueous maceration for extraction was employed as cold extract contains more tannins, cardiac glycosides and flavonoids and shows wider zones of inhibition because thermolabile components do not get destroyed (Abah and Egwari, 2011). Porwal *et al.* (2012) also reported that aqueous extract is said to be eco-friendly, non-toxic and safe.

The results highlighted that the yield percentage was higher of 24 hours (15%) than that of 12 hours (6%). The reason behind increased yield percentage with an increase in time period may be because more phytochemicals are extracted due to increase in keeping time.

The results are in alignment with the findings of Gupta, (2016) who reported increase in mass yield percent of *Syzygium cumini* (L.) extract with increased length of extraction period.

5.1.2 Analysis of Guava leaves extract concentration

Three different concentrations of Guava leaves extract were tried for its antibacterial property by agar well diffusion method. The results of the study indicated that the concentrations 1mg/ml and 3mg/ml showed moderate zones of 10mm and 12 mm, respectively, while 5mg/ml showed strong activity zone of 19mm against *Bacillus spp.* With an increase in concentration of Guava leaves extract the antibacterial property of Guava leaves extract also increased. The increase in antibacterial property with increase in concentration may be because of presence of more phytochemicals and more extract gets dissolved in higher concentration as compared to lower concentration. The results coincide with the results of Saini (2014) who reported that as the concentration of all treatments of *karanja* extracts increased percentage reduction of *Pseudomonas* and *Aspergillus* growth in all treated samples increased. The results are in line with the results of Hooda (2012) who reported that as the concentration of extract increased, percentage of bacterial reduction of herbal treated grey cotton and wool samples also increased. Gupta (2016) also revealed that the activity of the treated sample increased with the increase in extract concentration. Wasif and Ruble (2007) also reported that increase in concentration showed better zone of inhibition to bacterial presence as compared to lower concentration.

5.2 Effect of preparatory processes on physical properties of grey fabric

Desizing and scouring as a pre-treatment was given to selected grey cotton woven fabric. Desizing and scouring is important to remove the residual impurities present in intact fibrous substrate for better exhaustion of antibacterial ingredients into the fabric. After desizing and scouring there was significant increase in weight per unit area, thickness, bulk and fabric count both in warp and weft directions while there was significant decrease in tensile strength in both warp and weft directions and air permeability. After desizing and scouring, there was removal of starch and other impurities which resulted in compactness and closeness of weaves. The changes in physical properties of the fabric may therefore be attributed due to the compactness of the weave. The decrease in air permeability is also supported by Hooda (2012) who reported that air permeability of enzymatically scoured cotton fabric was found to be decreased.

5.3 Determination of bacterial resistance of Guava leaves extract treatment and its effect on physical properties

Determination of bacterial resistance of Guava leaves extract treatment on treated fabric was analysed against cellulose degrading bacteria i.e. *Bacillus spp.* quantitatively and its effect on physical properties was also observed.

5.3.1 Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.*

The assessment of efficacy of Guava leaves extract treatment against growth of *Bacillus spp.* was done at different incubation periods i.e. 0 day (soon after 24 hours), 7th day, 14th day, 21st day and 28th day of inoculation by manually counting the colonies. It was found that there was confluent lawn of growth of *Bacillus spp.* in controlled (desized and scoured) samples. The antibacterial treatment with 5g/l concentration of Guava leaves extract with dilution factor (10^7 and 10^8) was cent percent effective after 24 hours of inoculation of *Bacillus spp.* After 24 hours percent bacterial reduction gradually decreased and on 28th day it reduced to 67%. Thus, there was subsequent percent reduction in bacterial count with further increase in incubation period.

The antimicrobial activity of Guava leaves extract may be due to the presence of several phytochemicals as tannins, flavonoids etc. The attached herbs' extract disturb the cell membrane of the microbes through the physical and ionic phenomena (Sarkar and Munshi, 2003). The antibacterial assessment of the treated fabrics confirmed presence of active compounds such as tannin, eugenol and mallic acid etc. The results are very much in confirmation with the results obtained by Saini (2014) who reported that with an increase in the incubation period there was continuous increase in the bacterial colonies. Rajendran (2011) highlighted that there was percent reduction of 95.7 for *S.aureus* and 89.4 for *E. coli* in treated fabric against its respective control samples. The results are supported by the findings of Gupta and Laha (2007) who reported the good antibacterial activity of cotton fabric when treated with the tannin-rich extract of *Quercus infectoria* (QI) plant in combination with alum and copper against *Bacillus subtilis*.

5.3.2 Effect of Guava leaves extract treatment on physical properties of fabric

After the application of the Guava leaves extract treatment on cotton fabric the weight per unit area and bulk increased significantly while tensile strength in both warp and weft directions, flexural rigidity and air permeability decreased significantly. The changes in physical properties of the treated fabric may be because of the absorption, spreading and exhaustion of Guava leaves extract as antibacterial ingredient into the fibrous substrate.

The increase in weight per unit area and bulk of the treated fabric may be due to the absorption of the Guava leaves extract and swelling of the fibres. The increase in weight is similar to findings with the results of Hooda *et al.* (2013) who reported that weight add-on percent was increased as the concentration of extract increased.

The bulk of the treated fabric increased significantly. The increase in bulk was observed due to the increase in the fabric weight. The results are supported by the findings of Hooda (2012) who reported significant increase in the bulk of the scoured cotton and wool fabrics after the treatment with *Neem* and *Aloe vera* extracts applied for bacterial resistance.

Likewise, increase in bulk was reported by Saini (2014) after the application of *Karanja* extract on the samples treated with two different concentrations of 2.5g/l and 5g/l.

The decrease in tensile strength could be attributed due to the formation of intermolecular and intra-molecular cross links which reduces the possibility of equalizing the stress distribution, causing reduction in the capacity to withstand load, as well as prevents the movement of the fiber molecules causing severe tensile strength loss as reported by Sunder and Nalankilli (2012). Similarly, Gupta (2016) reported that the tensile strength of treated woven fabric decreased by 4.12 percent after the finishing treatment of *Syzygium cumini* (L.) leaves extract. The results are in line with Sathianayranam *et al.* (2010) who revealed that pomegranate extract cross linked with resin decreased the tensile strength by about 15 percent and that of *Tulsi* by about 13 percent in warp direction.

The decrease in flexural rigidity may be because of the swelling of the fibres due to take up of the treatment along with citric acid used during treatment. The results are in line with the results obtained by Gupta (2016) as she reported the decrease in flexural rigidity fabrics treated without resin cross linking agent and magnesium chloride in the presence of *Syzygium cumini* (L.) leaves extract, which indicated that the fabric have become pliable.

The decrease in air permeability may be attributed to compactness and closeness of weave due to entry in water bath and blockage of the pores of the treated fabric due to the size of herbal particles, which in turn leads to lower air permeability. Increased fabric thickness and more number of fibres per unit area offer more resistance to air flow leading to lower air permeability.

The decrease in air permeability after the application of treatment have also been reported by Hooda (2012), Saini (2014), Gupta (2016) and Chandrasekaran *et al.* (2013). Agne (2014) investigated air permeability of plain and plated knits and revealed that knitted samples after antimicrobial treatments have significantly lower air permeability comparing with untreated fabric.

5.4 Effectiveness of Guava leaves extract treatment with respect to washing and its effect on physical properties

Efficacy of Guava leaves extract treatment against washing was assessed by bacterial colony counts of *Bacillus spp.* after one wash on the 0 day (soon after 24 hours), 7th day, 14th day, 21st day and 28th day of inoculation for both the dilution factors i.e. 10^7 and 10^8 . The effect of washing on physical properties was also observed.

5.4.1 Effectiveness of Guava leaves extract treatment with respect to washing

The effectiveness of Guava leaves extract treatment one washing was analysed against growth of cellulose degrading bacteria i.e. *Bacillus spp.* All unwashed and washed samples were compared for growth of bacterial colonies. The Guava leaves extract treated samples exhibited cent percent wash durability after 24 hours of inoculation in washed

samples (one wash). After 24 hours it gradually decreased and on 28th day it reduced to 64%. Thus, there was continuous decrease in the antibacterial activity with an increase in incubation period after washing but even then the antibacterial treatment was actively retained in washed samples even on 28th day. After washing the treatments got washed from the surface due to weak forces between the antimicrobial agent and cellulosic material. This may be the reason for increased bacterial growth in the fabric samples after washing. Hence every wash needs to be renewed. The fabric samples need renewable herbal extract treatment after every wash.

Chandrasekar *et al.* (2013) studied the durability of bulk finished fabric and nanocomposite finished samples for *E. coli* and *S. aureus* after 1st, 5th and 10th wash which showed that the nanocomposite fabric was able to retain the antibacterial activity even after 10 industrial washes, thereby providing long term durability of the nanocomposite finished fabric.

Sathianarayanan *et al.* (2010) reported in case of *Tulsi* extract treated fabric, in each wash cycle the antibacterial activity decreased gradually and at the end of 15th wash cycle the percent reduction value reaches to 55 and 51.5 for gram positive and gram negative bacteria respectively.

Saini (2014) reported that *karanja* extracts in 2.5g/l concentration were very effective after 30 minutes of inoculation (100%) of *Pseudomonas* and *Aspergillus* after one wash. There was increase in bacterial colony with an increase in incubation period. Maximum (100%) percent reduction was observed in *Pseudomonas* growth upto 7th day. She also reported that it was needed to retreat the fabric samples after every wash.

Similar results were reported by Hooda *et al.* (2013) where they assessed the efficacy of Aloe vera finish on scoured wool fabric after different number of washing cycles and found that on 20 washing cycles, percentage reduction value decreased to 78.13% and 82.81 % for scoured wool fabric treated with 3g/l and 5g/l Aloe vera extracts, respectively.

5.4.2 Effect of washing on physical properties of fabric

It was evident that after washing there was significant decrease in weight per unit area, bulk, tensile strength in both warp and weft directions and flexural rigidity whereas significant increase in air permeability. The changes in physical properties may be because of removal of extract from the surface of fabric after washing.

The decrease in weight and bulk might be due to removal of Guava leaves extract as well as loose fibers. The results obtained are in alignment with the results obtained by Hooda (2012), Verma (2013), Saini (2014) and Gupta (2016) who reported the decrease in weight and bulk of the fabric with increased number of wash cycles.

The decrease in tensile strength and flexural rigidity might be due to the frictional action involved in washing as well as decrease in weight and thickness of the fabrics. These

results are again in line with the findings of by Hooda (2012), Verma (2013), Saini (2014), Sumithra (2015) and Gupta (2016) who reported the decrease in tensile strength and reduction in stiffness predominantly in the fabric when subjected to several washes.

Increase in the air permeability of cotton woven fabric was observed, after washing. Such an increase may be attributed due to the removal of Guava leaves extract and binding agent (citric acid) from the fabric surface, thus emptying the interfiber pores of cotton fabrics. Air permeability has a linear relationship with thickness of the fabric. It is noted that the decrease in thickness after different number of wash cycles also might have led to increased air permeability. The results are in line with the observations made by Karolia (2007), Saini (2015) and Gupta (2016) who reported that air permeability of the treated fabric increased after laundering.

5.5 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

The physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing were compared to analyse the effect of these treatments. Weight per unit area, thickness, bulk and fabric count in both warp and weft directions revealed that there was remarkable increase after the application of the Guava leaves extract treatment which decreased after washing. It is clear from the results that flexural rigidity, tensile strength in both warp and weft directions and bending length in both warp and weft directions reduced after the Guava leaves extract treatment and these were further decreased after washing. Changes were also found in air permeability and moisture regain of cotton woven fabric after treatment and washing. Both these properties decreased after the application of Guava leaves extract treatment but increased after washing. The changes in the physical properties were in close association with the results of Gupta (2016) and Saini (2014) who reported an increase in weight per unit area, thickness, bulk and decrease in flexural rigidity, tensile strength in both warp and weft directions, bending length in both warp and weft directions, air permeability and moisture regain after the application of treatment. They reported that an increase in air permeability and moisture regain was found after washing while decrease in weight per unit area, thickness, bulk and decrease in flexural rigidity, tensile strength in both warp and weft directions and bending length in both warp and weft directions was found after washing.

Serviceability of the textiles is the first requisite for the consumer. Bio-deterioration of textile fabrics by microbial action is very common problem under tropical conditions. Microbial growth on the textiles can be inhibited by applying chemical and natural botanical anti-microbial agents. Synthetic anti-microbial agents leads to fabric strength loss, make the fabric stiff, change the colour of the fabric and also harm the environment (Hussain, 2006). Many plant extracts from roots, stem, leaves, flowers, fruits and seeds of diverse species of plants exhibit anti-bacterial properties. Use of plant extracts for various textile treatments not only provides protection from environmental hazard but also safeguards the environment, prevent pollution and promote eco-friendly textiles. Use of such products also ensures the health benefits to the individual as well as the masses. Guava leaves which are powerhouse of phenolic compounds possess excellent antioxidant and antimicrobial properties. Keeping the above facts in mind the present study was conducted with following specific objectives:

1. To prepare the extract from leaves of Guava tree for application on cotton fabric.
2. To determine microbial resistance of the treated fabric
3. To study the efficacy of treatment on physical properties of the fabric.

Methodology

To achieve the objectives, different methodological procedures adopted for the present study were:

Cotton woven fabric of light to medium weight, suitable for apparel during summer was selected in the grey state and preliminary data of the fabric was studied by using standard test methods. The grey cotton woven fabric was subjected to desizing and scouring, for uniform application of treatment. Fresh, fleshy, mature and green leaves of Guava plant (Hisar *Surkha*) were collected. Aqueous extraction of shade dried and powdered leaves was carried out by cold maceration. The analysis of extract was conducted under two different parameters i.e. yield percentage and antibacterial property at three different concentrations. On comparative analysis the time period giving higher yield (24 hours giving 15%) and the concentration exhibiting highest zone of inhibition (5 mg/ml exhibiting 19mm) were selected and continued. Application of treatment was done on desized and scoured cotton woven fabric by exhaust method.

The efficacy of Guava leaves extract treatment against growth of cellulose degrading bacteria i.e. *Bacillus spp.* on controlled, treated and washed cotton fabric samples, was counted quantitatively by AATCC-I00 test method. The antibacterial activity of treated cotton woven fabric was compared to its controlled sample by calculating the percent reduction in

bacterial count. The samples were inoculated with *Bacillus spp.* separately. The observations were recorded soon after 24 hours of inoculation of *Bacillus spp.* on the same day and also on 7th, 14th, 21st and 28th days with dilution factors 10⁷ and 10⁸. Mean values of 10⁷ and 10⁸ dilution factors were calculated. Physical properties of controlled, treated and washed cotton woven fabric were studied. The efficacy of Guava leaves extract treatment and its effect of washing on physical properties of treated fabric was determined by subjecting the fabric samples to one wash.

Major findings

The major findings of the study are summarized under the following heads and sub heads:

- 6.1 Effect of preparatory processes on physical properties of grey fabric
- 6.2 Analysis and selection of Guava leaves extract concentration
- 6.3 Determination of bacterial resistance of Guava leaves extract treatment against growth of *Bacillus spp.* and its effect on physical properties
- 6.4 Efficacy of Guava leaves extract treatment with respect to washing and its effect on physical properties
- 6.5 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

6.1 Effect of preparatory processes on physical properties of grey fabric

The gain in fabric weight (7.01), fabric count both in warp (5.68) and weft (4.23) directions of fabric were found to be significant at 1% level of significance. While increase in thickness (4.54) and bulk (3.51) were found to be significant at 5% level of significance. The decrease in tensile strength both in warp (9.38) and weft (4.79) directions of the fabric was found to be significant at 1% level of significance. Whereas decrease in air permeability (3.14) was found to be significant at 5% level of significance. The changes in bending length in both warp (1.38) and weft (0.20) directions, flexural rigidity (1.24) and moisture regain (1.67) were found to be non-significant.

6.2 Analysis and selection of Guava leaves extract concentration

6.2.1 Determination of yield percentage of Guava leaves extract

Cold aqueous maceration was conducted at two different time periods i.e. 12 hours and 24 hours for extraction of compounds from Guava leaves. The extraction time period had an effect on yield percentage. The yield percentage of 24 hours was found to be higher (15 %) than that of 12 hours (6 %). Therefore, 24 hour time period for extraction was selected and continued for further research work.

6.2.2 Antibacterial property of Guava leaves extract at different concentrations

Antibacterial property of Guava leaves extract was assessed at three different concentrations. The antibacterial activity was found to increased with an increase in

concentration. Strong antibacterial activity was observed at 5mg/ml concentration with 19 mm of zone of inhibition. Therefore, 5 mg/ml concentration had a strong zone of inhibition i.e. 19 mm against *Bacillus spp.* was selected and continued for further research work.

6.3 Determination of bacterial resistance of Guava leaves extract treatment and its effect on physical properties

6.3.1 Efficacy of Guava leaves extract treatment against growth of *Bacillus spp.*

Guava leaves extract treatment with dilution factor 10^7 & 10^8 were found effective with 100% reduction on the same day after 24 hours of inoculation with 5gm/l concentration and also upto 7 day with 93% reduction.

6.3.2 Effect of Guava leaves extract treatment on physical properties of fabric

After Guava leaves extract treatment fabric weight (6.89) and bulk (6.32) of fabric were increased, which were found to be significant at 1% level of significance. While tensile strength both in warp (5.77) and weft (3.20) directions, air permeability (5.50) and flexural rigidity (3.55) were decreased. These changes were significant at 1% (tensile strength and air permeability) and 5% (flexural rigidity) level of significance. The changes in thickness (2.09), bending length both in warp (1.33) and weft (1.76) direction, fabric count both in warp (1.98) and weft (1.14) direction and moisture regain (2.52) were found to be non- significant.

6.4 Efficacy of Guava leaves extract treatment with respect to washing and its effect on physical properties

6.4.1 Efficacy of Guava leaves extract treatment with respect to washing

After washing the number of bacterial colonies increased. There was continuous decrease in percent reduction from 0 day to 28th day of inoculation. The percent reduction decreased from 100 % to 64% on 28th day.

6.4.2 Effect of washing on physical properties of fabric

After washing, there was increase in air permeability (4.36) which was found significant at 5% level of significance. The fabric weight (7.87) and bulk (9.72) were found to be decreased at 1% level of significance. While decrease in flexural rigidity (3.06) and tensile strength in warp (2.78) and weft (3.71) directions were found to be significant at 5% level of significance. The change in thickness (2.09), bending length both in warp (1.82) and weft (2.00) direction, fabric count both in warp (1.90) and weft (1.36) direction and moisture regain (1.00) were found to be non-significant.

6.5 Comparative analysis of physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing

An attempt has been made to compare the physical properties of cotton fabric after preparatory processes, Guava leaves extract treatment and washing to analyse the effect of these treatments. The results revealed that there was remarkable increase in weight per unit area (+5.67, +3.75), thickness (+13.38, +6.25), bulk (+4.64, +4.77) and fabric count both in

warp (+16.12, +5.55) and weft (+13.63, +4.00) properties after the application of the Guava leaves extract treatment while these properties i.e. weight per unit area (-4.13), thickness (-5.88), bulk (-9.44) and fabric count both in warp (-5.26) and weft (-3.84) were decreased after washing. The flexural rigidity (-2.68, -6.62, -8.6), tensile strength in both warp (-10.46, -9.00, -7.69) and weft (-7.62, -8.34, -9.82) directions and bending length in both warp(-5.11, -8.33, -8.02) and weft(-1.14, -7.55, -6.91) directions reduced after the Guava leaves extract treatment and continuously decreased after washing also. There was decrease in air permeability (-1.58 and -1.51) and moisture regain (-14.28 and -11.11) of cotton woven fabric after treatment and but after washing air permeability (+1.97) and moisture regain (+6.25) increased.

Conclusion

Extract of Guava leaves was observed as potential natural source for antibacterial activity. Understanding the importance and significance of cotton in day to day lives of Indian people and this being a comfortable habitat for most of the microbes, was used as a substrate for the application of Guava leaves extract. Guava leaves extract treatment applied with exhaust method with 5 gm/l concentration exhibited remarkable improvement in antibacterial properties of the treated fabric against cellulose degrading bacteria i.e. *Bacillus spp.* Though the treated fabrics showed good efficacy against washing however it is needed to be renewed after every wash for good resistivity to washing.

Recommendations

- *Psidium guajava (L)* is recommended for development of antibacterial textile.
- 5% concentration of Guava leaves extract is effective for development of antibacterial textiles.
- The treatment can be used at household level as renewable treatment for imparting resistance against *Bacillus spp.*

Guava leaves extract treated fabrics can be used for development of protective and medical textiles due to their good antibacterial property.

Limitations

- Only leaves of the Guava tree have been explored for antimicrobial activity.
- Cotton fabric has only been studied as a substrate, while wool and silk are also prone to microbial attack.
- Only one concentration 5g/l has been used for application.
- The antimicrobial activity of the extract was tested against only one gram positive and no gram negative bacteria or fungus.
- Extraction was conducted only by maceration and application was done only by exhaust method.

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ANNEXURE-I

Composition of Mery mandel medium:

| Composition | Amount |
|--|---------------|
| Potassium Phosphate (KH_2PO_4) | 2 g/l |
| Ammonium Sulphate ($(\text{NH}_4)_2\text{SO}_4$) | 1.4 g/l |
| Calcium chloride (CaCl_2) | 0.3 g/l |
| Peptone | 0.25 g/l |
| Yeast extract | 0.2 g/l |
| Ferrous Sulphate (FeSO_4) | 5 mg/l |
| Magnesium Sulphate (MnSO_4) | 5.6 mg/l |
| Zinc Sulphate (ZnSO_4) | 3.34 mg/l |
| Calcium Chloride (CaCl_2) | 2 mg/l |
| Tween 80 | 1 ml |

ANNEXURE-II

Composition of Nutrient agar medium:

| Composition | Amount (g/l) |
|--------------------------------|---------------------|
| Peptic digest of animal tissue | 5 |
| Sodium chloride | 5 |
| Beef extract | 1.50 |
| Yeast extract | 1.50 |
| Agar | 15 |

ANNEXURE-III

Composition of CMC (Carboxy methyl cellulose) agar medium:

| Composition | Amount (g/l) |
|--------------------|---------------------|
| CMC | 5.0 |
| Glucose | 20.0 |
| Yeast Extract | 5.0 |
| Agar- agar | 20.0 |

ABSTRACT

Title of Thesis : Effect of Extract of Guava Leaves on cotton for Microbial Resistance

Full name of the Degree holder : Zeba Jamal

Admission No. 2014HS22M

Title of Degree : Master of Science

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Degree awarding University : CCS Haryana Agricultural University, Hisar

Year of award of Degree : 2016

Major Subject : Textile & Apparel Designing

Total No. of pages in Thesis : 50 + iv + I

Number of words in the abstract : Approx 440

Keywords : Guava leaves extract, cotton, antibacterial, physical properties

Consumers have become more aware of hygiene and potentially harmful effects of microbes, which in turn have increased the demand of antimicrobial textiles. Considering the significance of antimicrobial textiles, the Guava leaves extract treatment was prepared and applied on cotton fabric. To find out the efficiency of Guava leaves extract treatment the parameters like antibacterial activity, wash durability and physical properties of controlled, treated and washed samples were compared. Extraction of Guava leaves was conducted by cold aqueous maceration for two different time periods. 24 hours showed yield percentage of 15 % which was greater than yield percentage obtained by 12 hours i.e. 6%. The concentrations 1mg/ml, 3mg/ml and 5mg/ml were tried for antibacterial activity against *Bacillus spp.* The 5mg/ml concentration exhibited strong zone of inhibition of 19 mm. The Guava leaves extract was applied on cotton woven fabric by exhaust method in 5g/l concentration. The antibacterial treatment with 5g/l concentration of Guava leaves extract with dilution factor (10^7 and 10^8) was cent percent effective after 24 hours of inoculation of *Bacillus spp.* After 24 hours percent bacterial reduction gradually decreased and on 28th day it reduced to 67%. The Guava leaves extract treated samples exhibited cent percent wash durability after 24 hours of inoculation in washed samples (one wash). After 24 hours it gradually decreased and on 28th day it reduced to 64%. Thus, there was continuous decrease in the antibacterial activity with an increase in incubation period after washing but even then the antibacterial treatment was actively retained in washed samples even on 28th day. After desizing and scouring fabric weight, thickness, bulk and fabric count both in warp and weft direction of fabric increased significantly while tensile strength in both warp and weft directions and air permeability decreased significantly. The percent changes in bending length in both warp and weft directions, flexural rigidity and moisture regain were found to be non-significant. After Guava leaves extract treatment fabric weight and bulk of fabric increased significantly while tensile strength in warp and weft directions, flexural rigidity and air permeability decreased significantly. The changes in thickness, bending length, fabric count in both warp and weft direction and moisture regain were found to be non-significant. After washing weight, bulk, flexural rigidity and tensile strength in warp and weft directions of Guava leaves extract treated samples decreased significantly while air permeability increased significantly. The changes in thickness, bending length, fabric count in both warp and weft direction and moisture regain were found to be non-significant. Conclusively, the Guava leaves extract treated fabrics exhibited excellent efficacy against antibacterial activity and washing. However, it is needed to be renewed after every wash for good resistivity to washing.

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Zeba Jamal

UNDERTAKING OF COPYRIGHT

I, **Zeba Jamal**, Admission No. **2014HS22M** undertakes that I give copyright to the Chaudhary Charan Singh Haryana Agricultural University, Hisar of my thesis entitled, **“Effect of Extract of Guava Leaves on Cotton for Microbial Resistance”**.

I also undertake that patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of Chaudhary Charan Singh Haryana Agricultural University, Hisar.

(Zeba Jamal)
Signature of the student